Research Article

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Lipid extraction of high-moisture sour cherry (*Prunus cerasus* L.) stones by supercritical carbon dioxide

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Abstract

BACKGROUND: Sour cherry (*Prunus cerasus* L.) stones are the major byproduct of the cherry industry and the efficient management of this biowaste can lead to achieving the food processing sustainability aimed at by the modern food industry. Despite its significant content of lipids, the valorization of cherry stone waste as feedstock for lipid extraction appears to be limited due to the high moisture content. This study explores the primary factors that affect the yield of lipid extraction using Soxhlet, Randall and supercritical carbon dioxide ($scCO_2$) extraction methods, with a particular emphasis on yield optimization for green extraction technologies ($scCO_2$).

RESULTS: The investigation revealed an increased lipid extraction yield for $scCO_2$ from 7.4 for dry crushed stones to 20.6 g per 100 g dry weight when the cherry kernels are separated. The high initial moisture content affected all three extraction methods, but mostly impacted the $scCO_2$ extraction, resulting in the co-extraction of an aqueous phase. Lipid and aqueous yield could be manipulated by time, temperature and pressure. However, no observable influence on the composition of fatty acid methyl esters was detected.

CONCLUSION: Numerous approaches are shown to enhance the lipid yield from cherry stone waste, depending on the desired outcome. When dealing with wet samples, Randall extraction proves to be the most effective method. On the other hand, $scCO_2$ extraction presents distinct advantages, such as the extraction of food-grade lipids and the co-extraction of a unique aqueous phase, which comes at the expense of a reduced lipid yield.

Supporting information may be found in the online version of this article.

Keywords: cherry pits; supercritical fluid extraction; extraction method comparison; water content; oil composition; biowaste valorization

INTRODUCTION

To tackle the ongoing issue of biowastes, byproducts and unused biomass, new ways for their recycling and valorization are continuing to be developed.[1,2] In this context, residual streams like sour cherry stones might be valorized to reduce the environmental impact related to their disposal and simultaneously to diversify the market possibilities by applying the main principles of the circular economy related to waste minimization. Moreover, in the context of increasing uncertainty related to the global political situation and the emergence of new producers, sour cherry (*Prunus cerasus* L.) full exploitation, for example bio-cascading, is aimed.

Sour cherries can grow in cold regions and are commercially available. Although sour cherries can be safely consumed directly, it is common to find them in the market as processed food, like juice, jams, marmalades and toppings, or as alcoholic beverages like cherry wine and cherry liqueur.[3,4] Sour cherries contain polyphenols, like phenolic acids, flavonoids and anthocyanins,[5,6] which are known for their antioxidative, anti-inflammatory and anticancer activities.[4,7-9] Sour cherries consist of skin, flesh, a stone (shell

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+ kernel) and a stem. For most sour cherry products, the juice of the cherries is the target. To obtain sour cherry juice, fresh sour cherries are pressed, and the desired liquid fraction is collected. The remains (skins, stems, flesh remains and stones) are considered as waste.[10-12] One potentially valuable fraction can be the cherry stones, which account for *ca* 15% of sour cherries' fresh weight.[13]

The yearly worldwide production from 2006 to 2016 reached about 1.1–1.3 million tons, mainly from Europe, with a corresponding amount of biowaste from stones estimated at between 165 000 and 195 000 tons.[14] Kandemir *et al.*[15] more recently reported that the world production of sour cherries was estimated at 1.4 million tons. Considering that most of the production is related to juice production, its impact is clear in terms of biowastes generated.

Several investigations focused on the valorization of cherry stone waste. Pollard and Goldfarb[16] proposed a conversion to biochar and activated biochar for soil amendments and heavy metal removal. Akalın *et al.*[17] chose the production of bio-oil through hydrothermal processing as a valorization route. A completely different approach was proposed by Grubesa *et al.*[18] considering cherry stones as aggregate in concrete. Despite these alternatives, the extraction of lipids remains the most consolidated and explored valorization method due to the high value of the fraction recovered.[5,10,13,19-21]

Yılmaz and Gökmen reported that 23% of the stone weight is accounted for by the kernel fraction, while the remaining 77% is accounted for by the shell.[13] Among the two, the kernel fraction is considered to have the highest lipid content; however, experimental data focusing on the comparison are lacking.

The most reported extraction strategies focus on classical extraction by nonpolar organic solvents[5,19,20,22-25] or extraction via supercritical carbon dioxide ($scCO_2$)[10,13,20,21] with and without the use of co-solvent. Although the application of classic organic solvent extraction is relatively simple, it has the disadvantage of a high environmental impact. Furthermore, difficulties in complete solvent recovery lead to contamination of the product and thus to reductions in quality and value of the product.[26,27]

An alternative to the traditional organic solvent extraction approach is extraction via supercritical fluids, for example with carbon dioxide. Extraction via $scCO_2$ is a promising method to reduce the environmental impact and avoid any solvent contamination in the product, which makes this technique especially suitable for food-oriented compounds.[28,29]

As with all unit operations, also the use of $scCO_2$ requires the optimization of the characteristic operating parameter of the process. In this way, the designer can target a specific objective function like the recovery of a target compound/class of compounds or the total yield.[21]

When comparing the lipid extraction yields from cherry stones or kernels corresponding to different extraction methods, only little data are given.[10]

The focus seems to be on the fatty acid methyl ester (FAME) composition of the transesterified extracts, which seems not to be significantly influenced by the choice of the extraction method.[13,19] Oleic acid (C18:1) and linoleic acid (C18:2) are reported to be responsible for 70–90% of the total fatty acid composition,[5,13,19,22,30] indicating a high unsaturated to saturated fatty acid proportion,[13] giving another argument for the recovery of lipids from this feedstock.

While other publications focus on a particular extraction technique and one specific raw material, the study reported here took a new approach that focused on the comparison of different extraction techniques and the influence of the starting material conditions. The primary objective of the study was therefore to explore the influence of parameters from the cherry feedstock and the choice of extraction method on the lipid yield and composition. Since $scCO_2$ is the most promising option in terms of sustainability and potential for optimization, its extraction parameters were additionally analyzed. Therefore, this paper provides a complete picture of the routes to be followed for high yield in lipid extraction.

EXPERIMENTS/MATERIALS AND METHODS

The cherry pomace used for the experiments was provided by cherry wine producer Frederiksdal located in the Danish region of Sjælland. The pomace was kept frozen at −22 °C until further use. For all samples the pomace was washed under running water to remove residues of flesh, sticks and stems from the stones. The stones were recovered in a sieve and dried using a paper towel.

During all experiments, the moisture content of the samples was continuously monitored and no change over time was observed.

Crushed cherry stones

After washing and drying, about 200 g of stones was ground in a GM 300 knife mill (Retsch, Haan, Germany) using 50 g of sample per batch. The grinding time was 2 min at a temperature of 22 °C. Afterward, all portions were mixed to ensure homogeneity and divided into four aliquots.

The first part constituted the sample called CS-Original and was used without any further treatment. The other parts were treated as follows: in a hot-air oven (UFE4000, Memmert, Schwabach, Germany) at 40 °C for 24 h (sample CS-D40); in a humidity chamber (HCP 108, Memmert, Schwabach, Germany) at 40 °C setting the relative humidity to 75% for 24 h (sample CS-RH75); or in a humidity chamber at 40 °C setting the relative humidity to 20% for 24 h (sample CS-RH20). All samples were stored at −20 °C in the dark until further use.

Separated cherry kernel

The kernel fraction was obtained by cracking the whole stone and sorting manually the kernels. This fraction was processed following the same procedure as for the crushed cherry stone samples. The untreated part was called SK-Original, while the others were designated SK-D40 (oven 40 °C, 24 h), SK-RH75 (humidity chamber 75%, 40 °C, 24 h) and SK-RH20 (humidity chamber 20%, 40 °C, 24 h) depending on the thermal treatment. All samples were stored at −20 °C in the dark, until further use.

Moisture analysis

For the dry weight determination, 2 g of cherry material was placed in a moisture balance (MB 160, VWR, Pennsylvania, USA). The balance recorded the initial weight and a halogen-infrared heat source set at 120 °C was used to evaporate the moisture from the sample. After the balance recorded no weight loss above 0.1% for 1 min, the remaining mass was used to calculate the loss of moisture. All measurements were carried out at least in triplicate.

Soxhlet extraction

An amount of 2 g of cherry material was accurately weighed into a lipid-free 22 mm × 80 mm thimble (Whatman, Maidstone, UK, pre-extracted with hexane) and closed with lipid-free cotton (Vernacare, Lancashire, UK, pre-extracted with hexane).

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The sample was then extracted for 5 h with 100 mL of hexane (technical grade isomers, Fisher Sci, Massachusetts, USA) for 25–30 cycles in a 70 mL Soxhlet extractor.

The extract was filtered and evaporated with an RV10 rotation evaporator (IKA, Staufen im Breisgau, Germany) at 40 °C at reduced pressure. Lipids were transferred with analytical grade *n*-hexane (3 × 0.5 mL; Acros Organics, Geel, Belgium) into a glass vial and dried until weight equilibration was achieved. Measurements were carried out at least in duplicate.

Randall extraction

An amount of 2 g of cherry material was accurately weighed into a lipid-free 26 mm × 60 mm thimble (Whatman, Maidstone, UK, pre-extracted with hexane) and closed with lipid-free cotton.

The sample was extracted with 100 mL of hexane in a Soxtec Avanti 2055 apparatus (Foss, Hilleroed, Denmark). A cooking step at 170 °C for 40 min and a rinsing step for 80 min at 170 °C were performed before the extract was concentrated to around 10 mL. The extract from the extraction cup was then filtered and transferred into a round-bottom flask, evaporated and transferred into a vial, similar to the reported Soxhlet extraction method, before weight determination took place. Measurements were carried out in triplicate.

Supercritical fluid extraction

A supercritical fluid extraction 500 apparatus (Separex, Champigneulles, France) was used with carbon dioxide (99.7%; Linde, Schiedam, the Netherlands) for all experiments. An amount of 50 g of cherry material was measured and placed in the extraction vessel of the unit. After 30 min temperature equilibrium time, the experiments were carried out with a CO_2 flow rate of 25 g min^{-1} for 6 h. Unless otherwise mentioned, the extraction was performed with a pressure of 350 bar and a temperature of 40 °C, which showed good performance in preliminary testing and other biomatrices.[20,21,31] The extract was collected every 30 min from the collection vessel (60 bar, 40 °C) and the carbon dioxide was continuously recycled.

For cleaning purposes, the vessel was depressurized and disconnected. The remaining unit was then flushed with carbon dioxide (25 g min^{-1}, *ca* 200 bar, 40 °C) for 5 min. This fraction was collected, and its weight was added to the total yield calculations as a cleaning fraction. Afterward, a 1 mL min^{-1} co-solvent stream of 96% ethanol (Arcos Organics, Geel, Belgium) was added to the $scCO_2$ for 10 min. This sample was collected independently and was not included in the weight determination. All experiments were carried out at least in duplicate.

Weight determination of lipid/water fraction

Most of the extracts recovered through $scCO_2$ consisted of two phases: a yellow lipid fraction and an aqueous fraction. Every sample was centrifuged for 3 min at 5000 rpm with a Universal 320 centrifuge (Hettich, Tuttlingen, Germany) at 22 °C.

After centrifuging, the test tube was weighed, then the upper oily fraction was carefully removed via Pasteur pipettes. The remaining aqueous fraction was weighed and the difference in weight before and after removing the lipid fraction was determined and used as lipid weight. After separation, all samples were stored at −20 °C in the dark until further use.

FAME determination

The FAME determination was based on transesterification as described by Lepage and Roy[32] and adapted according to Yu *et al.*[31] The internal standard (IS) solution was prepared by dissolving 40 mg of tetradecane (99%; Acros Organics, Geel, Belgium), 40 mg of methyl nonanoate (98%; Sigma Aldrich, St Louis, USA) and 40 mg of nonadecanoic acid (≥98%; Sigma Aldrich) in 10 mL of *n*-hexane (>99%; Acros Organics). Additionally, a FAME solution containing 20 mg of methyl palmitate (≥99%; Sigma Aldrich), 150 mg of methyl linoleate (≥99%; Sigma Aldrich), 120 mg of methyl oleate (Sigma Aldrich) and 10 mg of methyl stearate (99%; Sigma Aldrich) in 50 mL of *n*-hexane was prepared. Calibration standards were prepared by adding each of 0, 100, 250, 500 and 1000 µL of FAME solution to a vial before adjusting the total volume to 2.00 mL with *n*-hexane and adding 100 µL of the IS solution.

For the transesterification, about 5 mg of the lipid fraction sample was accurately measured in a glass sample tube, before 100 µL of IS solution was added. Then 4 mL of freshly prepared 5% acetyl chloride (98%; Acros Organics) in methanol (99.8%; Acros Organics) was added to each sample tube, before homogenization by vortexing. Afterward, the tubes were placed into a water bath at 60 °C and shaken every 15 min vigorously. After 60 min the tubes were cooled at ambient temperature and 1 mL of 5 mol L^{-1} NaCl solution (97%; Fisher Sci, Massachusetts, USA) in Milli-Q® water (Merck, Darmstadt, Germany) was added. Additionally, 2.00 mL of *n*-hexane was added to each tube, before the tubes were rotated for 1 h. As a last step, the tubes were centrifuged for 5 min at 5000 rpm and the upper organic layer was transferred into a GC-Vial for analysis.

An amount of 0.5 µL of the prepared organic layer was injected into a GC-FID 2030 (Shimadzu, Kyoto, Japan), which was equipped with a 30 m × 0.25 mm × 0.25 µm film thickness HP-5MS column (Agilent, Santa Clara, USA). The injection temperature was set to 300 °C and the split was adjusted to 1.20 mL min^{-1} column flow and 20 mL split flow. Hydrogen (6.0, Linde, Dublin, Ireland) was used as the carrier and detector gas. The detection temperature was adjusted to 325 °C.

The starting oven temperature was set to 80 °C and increased at 20 °C min^{-1} until 140 °C. From here the temperature was increased at 3 °C min^{-1} until 210 °C was reached followed by a temperature increment to 300 °C at 20 °C min^{-1}.

The quantification was performed by calculating the area of the desired FAME divided by the area of the IS methyl nonanoate and referring to the calibration curve with known concentrations.

Solvent-to-feed ratio

The solvent-to-feed ratio was calculated by dividing the mass of the solvent that came in contact with the sample by the sample mass, as reported in Eqn (1):

$$SF = \frac{m_{Solvent}}{m_{Sample}} \quad (1)$$

Method-specific normalized extraction efficiency

The method-specific normalized extraction efficiency was calculated for each method by dividing the yield per dry weight through the yield per dry weight of the driest sample of the chosen method (Soxhlet, Randall or $scCO_2$) and sample (crushed stone or separated kernel).

RESULTS AND DISCUSSION

Moisture content

Moisture content or moisture level is an important factor when it comes to extraction yield. To identify the influence of moisture

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level on the yield of different extraction methods, stones and crushed kernels were obtained at four different moisture levels. The results from the moisture analysis expressed in terms of moisture level are reported in Table 1.

The highest moisture level was found in the raw material as obtained from the producer. The moisture content of the untreated material was 28.8% for stones and 50.0% for kernels.

The driest samples were achieved by using a humidity chamber with a relative humidity of 20% for 24 h. Moisture levels of about 4.7% for stones and about 3.6% for kernels were observed. Oven drying at 40 °C yielded a moisture content of *ca* 10.5% for stones and 11.5% for kernels. A bigger difference in dry weight for crushed stones and kernels was observed for the case of 75% relative humidity. The moisture level was 17.7% and 33.3% for stones and crushed kernels, respectively. This result might be explained by the difference between crushed stones and crushed kernels in terms of pores, lipid content and initial moisture content.

Extraction methods

The samples were extracted by Soxhlet, Randall and $scCO_2$ (350 bar, 40 °C) methods and analyzed in terms of lipid yield per dry weight. The results are reported in Fig. 1. As a general trend, it was observed that the amount of total lipids extracted from kernel samples is higher than that extracted from crushed stones. The highest lipid yield per dry weight was achieved by Soxhlet extraction of the driest kernel sample (SK-RH20, 3.6%). The lowest yield was observed for $scCO_2$ extraction of the most moisturized crushed stone sample (CS-Original, 33.3%). The Randall and Soxhlet extractions offered a comparable lipid yield for samples with a moisture content below 33%. An evident trend emerged, where higher-moisture samples led to higher extraction yields with Randall extraction than with Soxhlet operation. This trend can be marked by comparing the Randall and Soxhlet extraction yield from the most moisturized sample (SK-original, 50.0%), in which only two-thirds of the former extraction yield was collected via the latter method. The gap between Soxhlet and Randall lipid extraction yield for highly moisturized samples might be explained by the additional cooking step used in the Randall extraction. The boiling enhances not only the lipid but also the water solubility.[33] Furthermore boiling creates local sheer stress due to the formation and cavitation of bubbles as well as an enhanced mass transfer. However, the highest lipid yield still corresponds to the Soxhlet extraction (SK-RH20, 3.6%).

When compared with Soxhlet and Randall extractions, the $scCO_2$ extraction showed a lower lipid yield in all tested cases. This might be due to the smaller solvent-to-feed ratio of 180 in comparison to *ca* 350 for Randall and 578 for Soxhlet (see supporting information). Besides this, Soxhlet and Randall extractions employ hexane as a solvent which has different solvation properties from carbon dioxide, for example density, dielectric constant, dynamic viscosity, polarity and physical state, contributing to the differences observed for the extraction yield.[33-35] Nevertheless, $scCO_2$ does offer a nontoxic and ready-to-consume product, absence of environmental contamination and easy downstream processing, which might compensate for the lower yield.[26,28,29]

The absolute values reported in Fig. 1 show a decrease in lipid yield per dry weight for increasing moisture content for all methods and biomasses. To investigate the effect of moisture on the different methods and samples, method-specific normalized lipid extraction efficiencies are shown in Fig. 2. A moisture range of 4–12% yielded minimal losses (>80% yield) for all methods and sample matrices. The relatively strong decrease in the yield of the separated kernel sample by Soxhlet extraction (Fig. 2(e)) is due to the high value of the extraction yield from the lowest moisture content, which is the reference point and not shown in the crushed stone matrix (Fig. 2(b)).

Further increase of moisture content in the crushed stone matrix to 17.9% resulted in an apparent efficiency loss for $scCO_2$, but not for Soxhlet and Randall extractions, indicating a higher sensitivity of the $scCO_2$ extraction process towards moisture than the other tested extraction processes. This trend was confirmed by the separated kernel matrix, which showed a similar trend for moisture content of 33.3%. The highest-moisture sample (SK-Original, 50%) was found to lead to only 29% of extraction efficiency, while Soxhlet extraction still resulted in 50% and Randall in 86% efficiency. Overall, Randall extraction was least affected by moisture content and always led to lipid yields of over 80%.

FAME composition

The lipid fraction obtained by Soxhlet, Randall and $scCO_2$ extractions from the separated kernel matrix was transesterified and analyzed for its FAME composition. The results are reported in Table 2. Methyl linoleate was found to be the most abundant FAME, accounting for around half of the total lipid fraction, followed by methyl oleate which accounted for roughly one-third of the total lipid fraction. The remaining fraction is mainly dominated by methyl palmitate and only a minor fraction by methyl stearate.

A conducted multivariate analysis of variance showed that the moisture level has no influence on the lipid composition (see supporting information). Furthermore, three of the four FAME components were also not significantly influenced by the choice of extraction method. The only significantly influenced component was methyl stearate, which showed a slightly higher average lipid yield for the Soxhlet extracts than for the other two methods. However, methyl stearate accounts only for 2% of the lipid content and its influence on the choice of extraction method is therefore limited.

Also investigated was whether the extraction process favors certain lipid compositions, which means that different FAME

Table 1. Moisture level of different cherry samples: crushed cherry stones (CS) and separated kernel fraction (SK)

	Crushed stones		Separated kernel	
Drying method	Name	Moisture level (± standard deviation) (%)	Name	Moisture level (± standard deviation) (%)
—	CS-Original	28.8 ± 0.3	SK-Original	50.0 ± 0.2
Humidity chamber 75%	CS-RH75	17.7 ± 0.4	SK-RH75	33.3 ± 0.5
Oven 40 °C	CS-D40	10.5 ± 0.4	SK-D40	11.5 ± 0.4
Humidity chamber 20%	CS-RH20	4.7 ± 0.3	SK-RH20	3.6 ± 0.2

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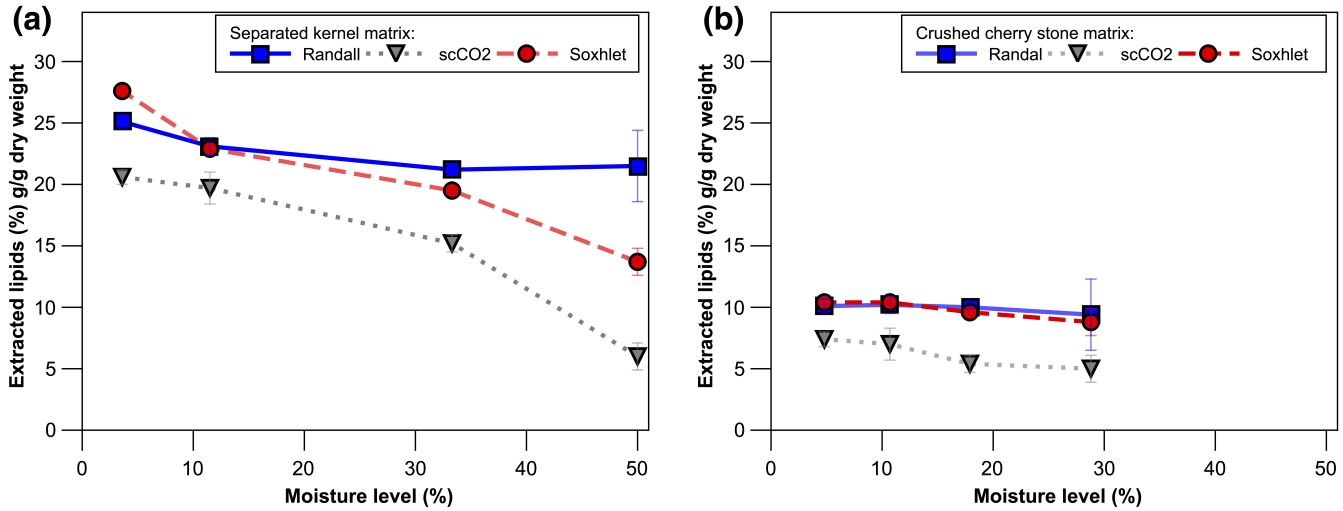

Figure 1. Lipid extraction yields from separated kernels (a) and cherry stones (b) obtained by Randall (blue, hexane 40 min cooking, 80 min rinsing), $scCO_2$ (grey, 350 bar, 40 °C, 6 h) and Soxhlet (red, hexane, 5 h) extractions as a function of sample moisture level. The results of yield are expressed in percentage of g g^{-1} dry weight.

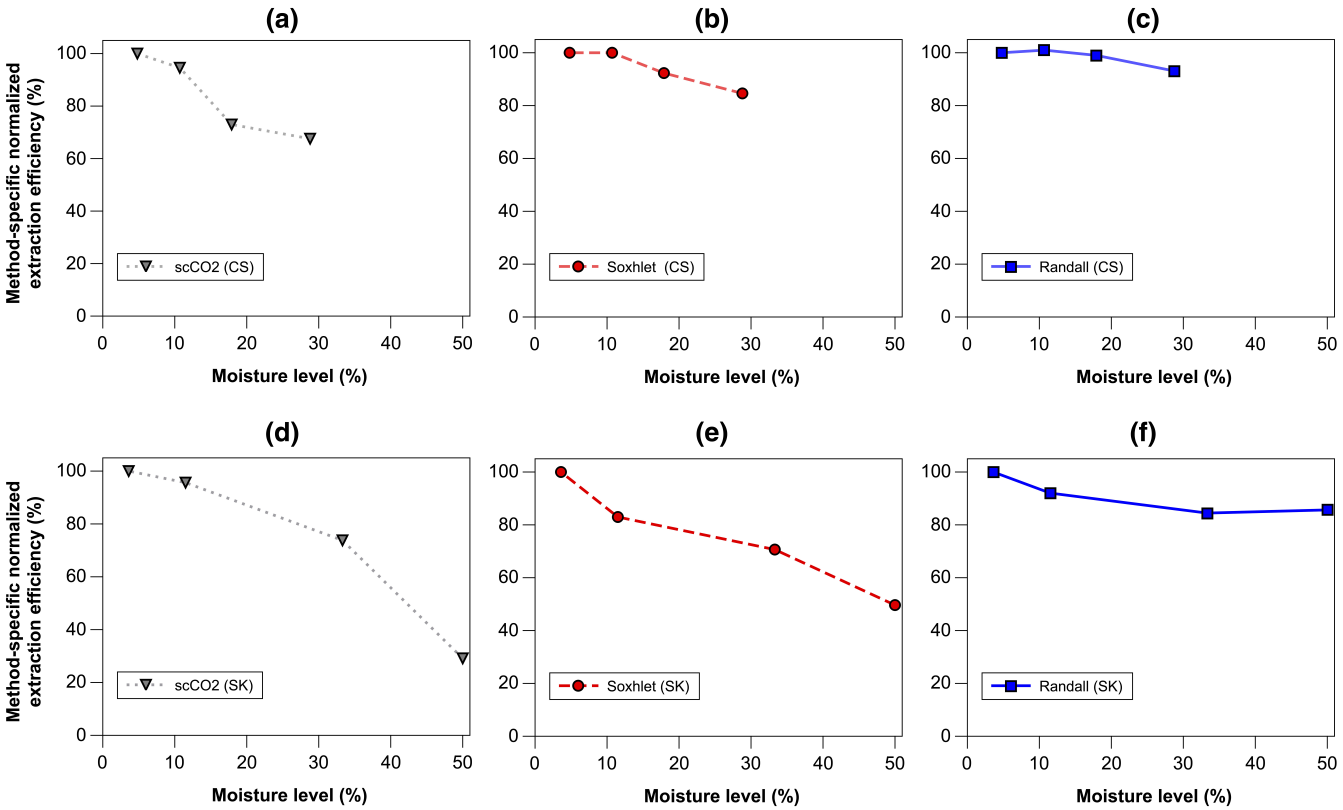

Figure 2. Method-specific normalized extraction efficiency against sample moisture level. Extraction efficiency of lipid yield per dry weight for crushed stones (CS) (a–c) and separated kernels (SK) (d–f) via $scCO_2$ (grey), Soxhlet (red) and Randall (blue) extractions.

compositions were observed at the beginning of the experiment from those at a later point of the experiment. However, such behavior was not found; in contrast to other matrices,[31,36] the FAME composition kept the same proportions over the total extraction time (see supporting information, S1). Other FAMEs were detected but were not quantified, due to their limited presence. The FAME composition obtained in this study indicates a high unsaturated to saturated fatty acid proportion in agreement with the results reported by Bernardo-Gil *et al.*[19]

Co-extraction of an aqueous phase with $scCO_2$

It was discovered that, differently from the Soxhlet and Randall extractions, for most $scCO_2$ extractions it was possible to collect an aqueous phase. The proportion between the lipid and aqueous

Table 2. FAME composition of esterified kernel lipid fractions

Sample (moisture content)	Extraction process	Methyl palmitate (C16:0) (%)	Methyl stearate (C18:0) (%)	Methyl oleate (C18:1) (%)	Methyl linoleate (C18:2) (%)	Total (%)	USF/SFA
SK-Original (50.0%)	Soxhlet	6	2	30	41	79	9
	$scCO_2$	5	2	33	47	87	11
	Randall	5	2	30	35	72	9
SK-RH75 (33.3%)	Soxhlet	5	2	30	44	81	10
	$scCO_2$	5	2	32	49	98	11
	Randall	5	2	31	44	82	13
SK-D40 (11.5%)	Soxhlet	5	2	30	44	81	11
	$scCO_2$	6	2	36	55	99	12
	Randall	5	2	33	47	87	11
SK-RH20 (3.6%)	Soxhlet	5	2	31	47	86	10
	$scCO_2$	5	2	32	49	88	12
	Randall	5	2	32	51	89	12

Lipid fraction was obtained from Soxhlet (hexane, 5 h), $scCO_2$ (350 bar, 40 °C) and Randall (hexane, 40 min cooking, 80 min rinsing) extracted at different moisture levels and analyzed in duplicate. Percentages refer to the total lipid weight fraction (w/w). USF/SFA was calculated by dividing the unsaturated fatty acid (USF) by the saturated fatty acid (SFA) content.

Table 3. Aqueous and lipid yield of $scCO_2$ extractions

Sample (moisture content)	Lipid g g^{-1} FW (%)	Lipid g g^{-1} DW (%)	Aqueous g g^{-1} FW (%)	Sample (moisture content)	Lipid g g^{-1} FW (%)	Lipid g g^{-1} DW (%)	Aqueous g g^{-1} FW (%)
CS-Original (28.8%)	3.6	5.0	13.6	SK-Original (50.0%)	3.0	6.0	16.8
CS-RH75 (17.9%)	4.4	5.4	4.8	SK-RH75 (33.3%)	10.2	15.2	15.7
CS-D40 (10.7%)	6.3	7.0	4.6	SK-D40 (11.5%)	17.4	19.7	7.5
CS-RH20 (4.8%)	7.0	7.4	0.8	SK-RH20 (3.6%)	19.8	20.6	3.6

Yield of the obtained lipid and aqueous phase of $scCO_2$ extractions after 6 h (350 bar, 40 °C) expressed in percentage of extracted lipid mass divided by either fresh weight (FW) or dry weight (DW) at different moisture levels.

phases is reported in Table 3. To the best of our knowledge, there has been no study of the extraction or analysis of an aqueous phase recovered from cherry stones or kernels.

As expected, the highest aqueous phase yield was obtained by $scCO_2$ extraction of the sample with the highest moisture content (SK-Original, 50.0%). This sample also showed the lowest yield for lipid extraction. It was found that a high moisture content resulted in a high aqueous yield and low lipid yield and vice versa.

In addition to the reported total yield of lipids with $scCO_2$, the extraction progress was monitored in terms of lipid and aqueous yield over time for the cherry kernel extractions.

$scCO_2$ lipid and aqueous extraction yield over time

The curve shape of extracted lipid per fresh weight (Fig. 3(a)) and extracted lipid per dry weight (Fig. 3(b)) differs from that of the aqueous phase yield per fresh weight (Fig. 3(c)) over time. The lipid extraction curves (Fig 3(a),(b)) of potentially high-lipid-yielding samples (SK-D40 and SK-RH20) seem to have a saturated shape which is shown by their potential high slope at the beginning, and their flat end. The saturated shape results probably through the limited mass transfer of lipids from the pores of the biomatrix to the $scCO_2$ phase, which was also reported elsewhere[20] and is in agreement with results for other lipid-containing matrices.[31] While the yield limitation for the lipid extractions for drier samples might result from a limited diffusion process (SK-RH20, SK-D40), the limitation for samples with higher moisture content (SK-RH75, SK-Original) might result from blockage of pores through water or inhibited partition of lipids of the $scCO_2$ phase. An increased moisture content showed therefore smaller lipid extraction rate per fresh and dry weight, which is not related to the maximum solubility of lipids inside the $scCO_2$ stream as shown in Fig. 3(a).

Differently from the lipid extraction yield, according to Fig. 3 (c), the profiles of the aqueous phase yield have a more linear behavior, as described for zero-order kinetics. This behavior is also reported by Brown *et al.*[37] for the dehydration of carrots via $scCO_2$ in similar conditions as here shown and is mostly influenced by the temperature (as discussed later). Samples SK-RH75 and SK-Original show similar aqueous extraction yield curves per fresh weight despite the different moisture content. This might indicate a limit of water solubility in the $scCO_2$ phase at the tested conditions, which could reduce the lipid uptake inside the $scCO_2$–water stream and lead to the decreased lipid extraction. Although literature about the theoretical maximum solubility of the bi-phase water–$scCO_2$ system is available,[38-40] a concrete value cannot be accurately determined, due to the co-interaction of cherry material and lipids, which might limit the water dissolution into the $scCO_2$ phase. This can be seen in sample SK-D40 (11.5%), where the water extraction trend emerged after around 150 min, corresponding to the

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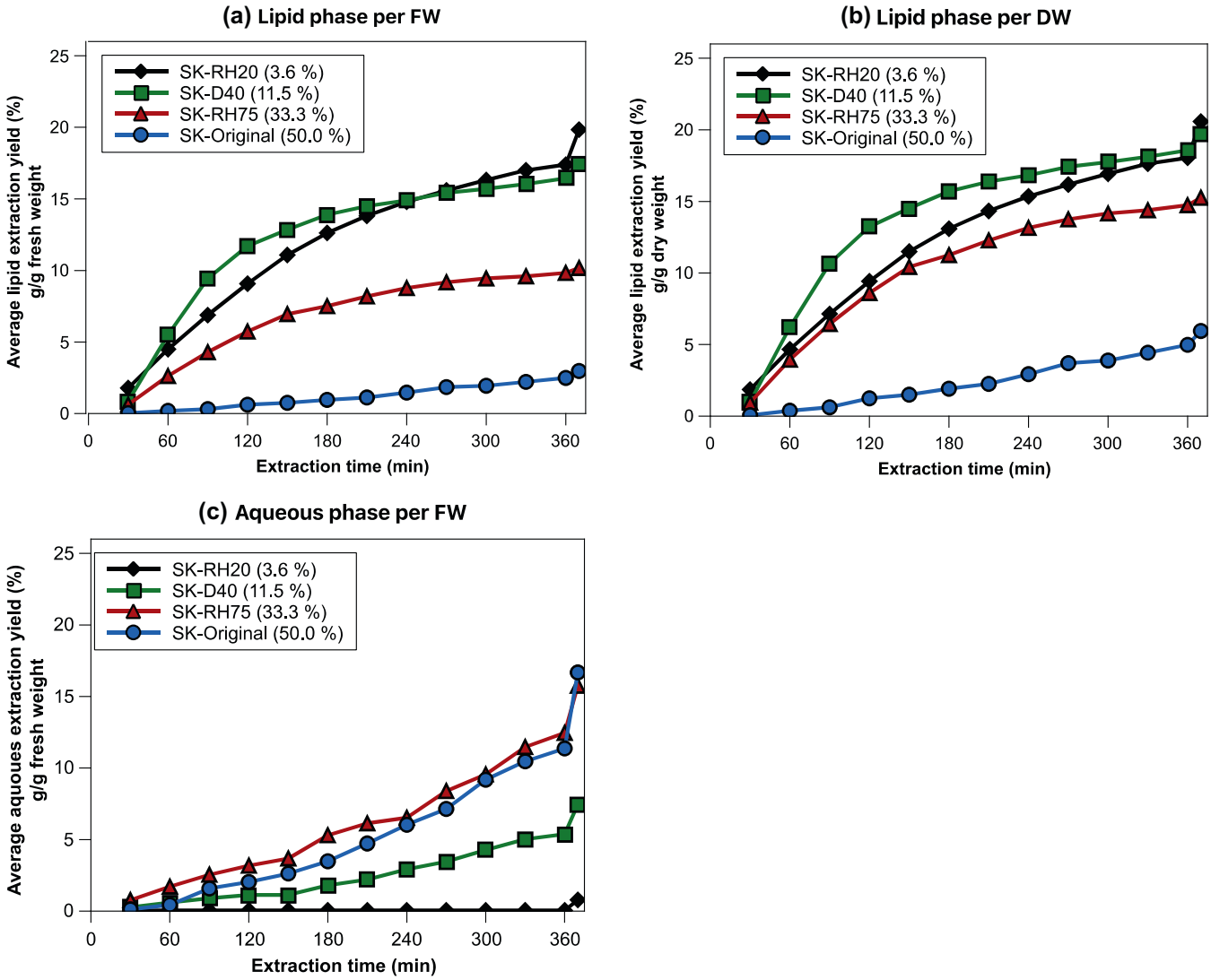

Figure 3. Lipid and aqueous extraction over time via $scCO_2$ extraction. (a) Accumulated yield of lipid phase per fresh weight (FW), (b) lipid phase per dry weight (DW) and (c) aqueous phase per fresh weight for separated cherry kernels at different moisture levels via $scCO_2$ extraction at 350 bar and 40 °C. The cleaning fraction, after 360 min of extraction, was considered and added as the data point at 370 min, which refers to the total yield as presented in Table 3.

point where around three-quarters of the lipids were extracted (Fig. 3(b)).

This behavior was not observed in the lower-moisture-containing samples. It could therefore be speculated that with longer extraction times the higher-moisture sample (SK-Original, 50.0%) could achieve a higher lipid extraction rate since most of the water is extracted, and the pores might be free to be accessed by the carbon dioxide.

During the extraction of the driest sample (SK-RH20), the highest lipid yield was achieved, and almost no aqueous phase was present and is therefore recommended for processes aimed at lipid yield, since it also avoids the need for another separation step.

Parameters influencing lipid or aqueous $scCO_2$ extraction

Due to the high lipid and medium aqueous yield, the crushed stone sample CS-D40 was chosen to be further analyzed for extraction at different conditions of pressure and temperature. Although the cherry kernel sample (SK-D40) resulted in a higher lipid fraction than the crushed stone sample (CS-D40), the latter was chosen concerning the ease of handling and economic potential. Sample CS-D40 with a moisture content of 10.7% was extracted with similar time (6 h) and flow rate conditions (25 g min^{-1} CO_2) but for a pressure of 150, 350 and 550 bar and a temperature of 40, 60 and 80 °C. The influence of pressure and temperature was examined with respect to the accumulated lipid and aqueous extraction yield. The effect of temperature and pressure on the accumulated yield of aqueous and lipid phases for a 360 min $scCO_2$ extraction is shown in Fig. 4.

The highest pressure (550 bar) and highest temperature (80 °C) resulted in the highest measured lipid yield (7.0 g per 100 g of initial sample). This result would increase the extraction yield from 67% to 74% in comparison to the Soxhlet operation (Fig. 1) and shows that the co-extraction of an aqueous phase does not necessarily limit the extraction of the lipids.

The overall dominating effect for the extraction of lipids in the investigated parameter frame appears to be pressure. However, experiments at the lowest constant pressure (150 bar) showed a huge difference in lipid yield, although similar pressure was

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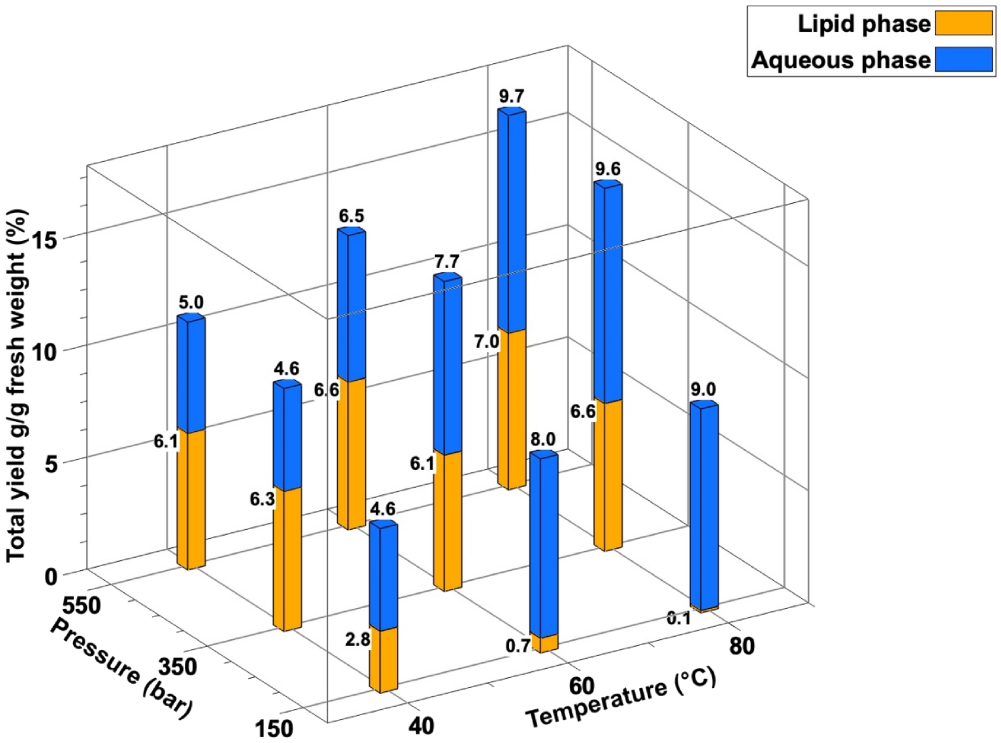

Figure 4. Influence of pressure and temperature on $scCO_2$ extraction yield. Accumulated yield of lipid (yellow) and aqueous phase (blue) after 6 h of extraction with $scCO_2$ at different temperature and pressure conditions.

applied. This can be explained by the density change of $scCO_2$ (see supporting information).[41] This means that pressure becomes the dominating parameter for the extraction of lipids only after a certain threshold of density is reached.

The greatest aqueous yield was realized by 550 bar at 80 °C, followed by 350 bar at 80 °C and 150 bar at 80 °C. The data demonstrate a positive correlation between aqueous yield and temperature, meaning that the higher the temperature the more water can be extracted. It was found that a change in pressure has no significant effect on the extracted aqueous phase. This result is in agreement with binary solubility data of water and $scCO_2$[38,39] and was also found for other biomatrices, for example carrots[37] and apples.[42]

CONCLUSION

The study reported demonstrates the influence of the choice of extraction method, choice of raw material, raw material condition and extraction parameters on the lipid yield of cherry material, a previously considered waste product. While earlier studies have primarily focused on optimizing specific values or parameters of a chosen extraction method, the present study places a greater emphasis on the raw material.

The separation of kernels from cherry stones emerged as the most critical factor, leading to a doubling or even greater increase in terms of yield. This was followed by factors like moisture level and choice of extraction method. The findings reveal that the highest lipid yield can be achieved with the driest kernel samples using Soxhlet extraction, although the difference from Randall extraction was relatively small. However, Randall extraction was found to be rather unaffected by moisture level, due to the application of boiling hexane.

$ScCO_2$ extractions (350 bar, 40 °C) were inferior in lipid yield to the other two conventional extraction methods, and it was observed that $scCO_2$ extraction suffers the most due to increased moisture content. However, it offers a contamination-free and food-grade product, which might compensate for the potential lower yield.

The lipid yield of $scCO_2$ extraction was improved by elevated temperature (80 °C) and pressure (550 bar) values. The FAME composition was analyzed, and it could be shown that the composition is not or only to a limited extent influenced by the choice of extraction method, moisture content, pressure, temperature or time. The demonstrated high ratio of unsaturated to saturated FAMEs underlines the importance of the exploitation of cherry stones and green extraction techniques such as $scCO_2$.

Among the contamination-free and food-grade lipid extracts, the $scCO_2$ extraction offers a great potential for the co-extraction of a novel aqueous phase, which could contain valuable phenolic components and is mostly influenced by temperature. Therefore, this work promotes the valorization of unused biomasses and helps to reduce waste.

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DATA AVAILABILITY STATEMENT

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

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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