


Article

Novel Sustainable Alternatives for the Study of the Chemical Composition of Cork

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Abstract: Cork is a natural and renewable material extracted from the cork oak *Quercus suber* L. (1) Background: The cellular structure and chemical composition of the plant wall give cork its physical and mechanical properties. Chemically, the composition of cork is principally dominated by the presence of suberin as the main structural cell wall component and affected by its close association with other components. The usual chemical analysis of cork has been partially adapted from wood and other lignocellulosic analytical methods and it is a method with extensive procedures. This study aims to (i) find quicker and more sustainable methods for testing the cork chemical composition, (ii) conduct extraction tests using alternative techniques, (iii) optimize alternative methodologies, and (iv) validate the proposed sustainable methodologies. (2) Methods: Microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) are explored as alternative systems to the traditional method. The percentages of the extractives obtained via MAE are lower than the percentages obtained by means of the classical composition. (3) Results: However, the percentages of suberin are very similar in both methods: 46.39% in the case of the classical composition and 45.11% in the case of microwave-assisted extraction. No significant differences are observed between the content of the extractives in the dichloromethane, ethanol, and water obtained via ASE and the results obtained with the classical methodology. (4) Conclusions: MAE and ASE are faster methods; they use less solvents and provide more reproducible results than the classical chemical composition methodology. These data pave the way for novel sustainable alternatives relative to the studies of the chemical composition of cork.



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Keywords: cork; chemical composition; cork extractive; Soxhlet; ASE; MAE

1. Introduction

Cork comes from the outer bark of the cork oak *Quercus suber* L. Cork is a natural and renewable material extracted mainly from the Western Mediterranean area. Cork oak forests contribute to different environmental services, such as carbon dioxide fixing, hydrology regulation, the prevention of desertification, and the preservation of wildlife [1]. The entire cork supply chain, spanning from the forest to the end customer, relies on the continuous and sustainable production of cork. Consequently, it hinges upon the effective and responsible stewardship of cork oak forests. These forests are a model of balancing environmental preservation and sustainable development.

Cork's cellular structure and the chemical composition of the plant's wall (suberin, lignin, and polysaccharides) give cork its physical and mechanical properties [2]. Chemically, the composition of cork is dominated by the presence of suberin as the main structural cell wall component and by its close association with other components [2]. This requires a difficult methodological approach in order to entirely analyze cork in terms of its chemical components. The study of the composition of cork began in 1787 by Brugnatelli [3]. From the end of the 18th century to the present day, the micromolar structure of cork and its chemical composition are still being studied, and there are still no clear models. The results

of the chemical composition can be determined by different factors: the methodology used, the cork's origin, the physiological state of the tree, or the number of specimens. The structural components of the cork cell wall are as follows: suberin (50% approx.); lignin (20–25%); polysaccharides (about 20%), such as cellulose and hemicellulose; the extractive (14–18%), which includes lipids and phenolic substances; and inorganic components (1–2%), presented in the order of relative importance [3,4] (Figure 1).

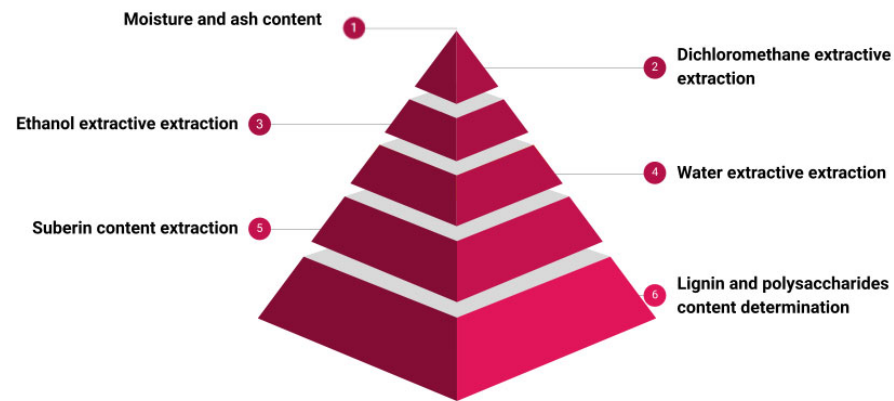


Figure 1. Cork chemical composition analysis diagram, sequential determination. Source ICSURO.

The usual chemical analysis of cork has been adapted from wood and other lignocellulosic analytical methods with respect to the determination of ashes, extractives, lignin, and holocellulose. The conventional approach to the chemical characterization of cork is to report its summative chemical composition. The analysis of the chemical composition of traditional cork is based on a sequential determination [5] of the ash contents (TAPPI 211), extractives (TAPPI 204/207/) [6], suberin [7], Klason lignin and acid-soluble lignin [8], and holocellulose (TAPPI 222/250).

Thus, the traditional chemical analysis method of cork is based on a three-step serial extraction using the Soxhlet [3]. The procedure begins with using a Soxhlet extractor with an organic solvent that has apolar characteristics (dichloromethane) for the directed extraction of compounds of a ceroid nature for 6 h. It is followed by a second Soxhlet extraction with alcohol that lasts 8 h, and finally, a Soxhlet extraction with water takes place for a duration of 16 to 24 h; alternatively, an extraction with a methanol and water solution can also be performed. With these extractions, most polyphenols are extracted. With a sample free of extractives, the suberin contents are determined based on alkaline methanolysis (3 h), followed by acidification and rotary evaporation (1 h), resuspension with two solvents, and decantation to obtain suberin in the organic phase (1 h). With the suberin-free fraction, the determination of the holocellulose content via acidification or the determination of lignin contents via acid hydrolysis is performed [5].

The total time for extractive determination and the analysis of the suberin content is at least 44 h. Therefore, the methodology requires very long durations and the use of different solvents and techniques, making its implementation as a routine quality test in cork manufacturing difficult. The cork sector comprises two basic systems: the forestry system and the industrial system, which is the system in which the raw materials are turned into different products [1]. The properties of cork are related to its chemical composition and the macromolecular structure of its components. A quick and easy cork chemical composition test is of interest to the cork sector. Both the forestry cork and industrial cork systems have a decision-making process that is based on cork quality. The destination of the highest quality cork is the cork stopper industry [3]. The cork sector is based on the production of cork stoppers, and stoppers comprise the cork product that produces the most economic return to the cork sector. The correct classification of cork quality therefore provides increased economic performance. Exploring new technologies that optimize the chemical composition tests of cork can allow for the cork industry to verify the quality

of cork and direct its destiny. For this reason, a faster and more efficient methodology is needed.

New technologies have emerged as potential tools that can reduce the amount of solvent and treatment times and increase reproducibility and energy consumption, thus improving the recovery of valuable compounds with lower costs for chemical analysis [9]. Methodologies based on newer technologies have been explored, such as microwave-assisted extraction (MAE) or accelerated solvent extraction (ASE) systems (Figure 2).



Figure 2. Comparisons of classical extraction methodologies (Soxhlet) with new alternatives: microwave-assisted extraction (MAE) or accelerated solvent extraction (ASE) systems (from left to right). Source ICSURO.

On the one hand, MAE is a promising green extraction method based on electromagnetic radiation with frequencies from 0.3 to 300 GHz. The rapid energy supply is transformed by quickly heating the solvent and suspension. This treatment is homogeneous and efficient for extraction. MAE is based on the direct effect of ionic conduction dipole rotation on molecules. When MAE is applied, the absorption of energy in the plant matrix and especially by polar molecules, such as water (moisture content), leads to cell rupture, which facilitates the recovery of the molecules of interest. Furthermore, the increase in extraction yields under microwave irradiation may be due to a synergistic combination of two transfer phenomena: mass and heat working in the same direction [9]. On the other hand, ASE is a sample preparation technique that uses solvents at elevated temperatures and pressures. Elevated temperatures increase the removal efficiency of analytes from the matrix. The increase in pressure keeps the solvent in a liquid state, even when the temperature is above the boiling point. It improves the diffusion of the analyte in it, rendering the extraction faster and more efficient. ASE can be used to automate the process, mitigate variability, and ensure reproducible results [10].

Sustainable practices rooted in green chemistry offer a promising avenue for the cork industry, exemplifying an integration of environmental responsibility and industrial innovation. Green chemistry principles emphasize the design and utilization of chemical processes that minimize environmental impact, promoting the efficient use of resources and a reduction in hazardous substances [11]. The scenery adorned with cork oaks serves as a prime illustration of achieving harmony between conservation and progress, ultimately benefiting both humanity and the environment. This man-made ecosystem is the outcome of centuries of continuous oversight and purposeful silviculture endeavors, shaping both cork oak trees and their surrounding environment. Through practices like grazing, clearance, or plowing for agricultural purposes, cork oak forests underwent a gradual transformation to expansive woodlands, exemplifying the dynamic interplay between human

activities and the evolution of these ecosystems. According to the inner sustainability of the cork sector, the chemical characterization of cork, crucial for quality assessments, can be approached with sustainable techniques, ensuring accurate results without compromising environmental integrity.

The aims of the present study are as follows: (i) identify quicker and more sustainable methodologies for cork chemical composition testing, (ii) carry out extraction tests using alternative techniques, (iii) optimize alternative methodologies, and (iv) validate the proposed or sustainable methodologies.

2. Materials and Methods

2.1. Cork Samples

Cork samples for the optimization study comprised 5 kg of 3–7 mm by-product supply by El Trust Group OLLER (Cassà de la Selva, Spain). The whole sample was ground with a ZM-200 ultra centrifugal mill (Retsch, Haan, Germany) and coupled to 0.45 mm ring-meshed sieves, and it was subsequently filtered with a sieve shaker (CISA, Barcelona, Spain) to obtain a granulometric fraction of 0.45 to 0.70 mm. Then, the sample was stored under dry and dark conditions in a drying and sterilization oven (DIGITHEAT TFT 52 L 1.00, Auxilab, Beriáin, Spain) at 37 ± 3 °C for a minimum of 24 h prior to the process to preserve constant humidity.

2.2. Reagents

Dichloromethane, ethanol, methanol, and chloroform were all of super purity grade, and they were obtained from Romil (Cambridge, UK). A 25 wt% sodium methoxide solution in methanol was purchased from Sigma-Aldrich (Merck, Darmstadt, Germany); sulfuric acid 95–97% AGR, ISO, and ACS were from Labkem (Barcelona, Spain); and deionized distilled water was from Adesco (Granollers, Spain).

2.3. Accelerated Solvent Extraction

An accelerated solvent extraction system, ASE 200, equipped with a solvent controller unit from Dionex Corporation Thermo Scientific (Sunnyvale, CA, USA), was used to perform extractions with the three different solvents. Extractions were carried out at three different extraction times, and two samples and two different extraction temperatures for each solvent were used according to the above experimental design. Tests were also carried out using serial extractions for the same solvent. Likewise, all extractions were performed in 100 mL extraction cells. Prior to each experiment, an extraction cell was pre-heated for 5 or 6 min depending on the extraction temperature. As described in Herrero M. et al. 2004 [12], the extraction procedure has 7 steps: (1) The sample is loaded into the cell; (2) the cell is filled with solvent up to a pressure of 1500 psi; (3) the initial pre-heating period takes place; (4) a static extraction with all system valves closed is performed (process known as extraction time); (5) the cell is eluted and rinsed (with 50% cell volume using the extraction solvent); (6) the solvent is purged from the cell with N₂ gas; and (7) depressurization takes place. Between extractions, a rinse of the complete system with the same solvent used for the extraction was carried out in order to prevent any extracts from being carried over. During the process, the equipment can gradually release extracts to maintain the pressure. All extracts were collected in 250 mL bottles. For solvent evaporation, a Rotavapor Laborota 4001-efficient (from Heidolph, Schwabach, Germany) device was used when the extracts were obtained with dichloromethane and ethanol, and for the case of the water extract, an evaporating dish in a MultiMix Heat D hotplate (from OVAN, Barcelona, Spain) was used under laboratory fume hoods. After evaporation, the extracts were placed in a drying oven at 60 °C O/N (DIGITHEAT TFT 52 L 1.00). The total content of dry extracts was recorded up to a constant weight. The parameters that have the greatest influence on the extraction via ASE were selected using a factorial design.

2.4. Microwave-Assisted Extraction

Microwave-assisted extraction (MAE) experiments were carried out using a CEM Discover LabMate Microwave Reactor (Discover SP[®] CEM Corporation, Matthews, NC, USA). The CEM Microwave Synthesizer supports a range of volumes from 5 mL to 125 mL, with a selectable power output of 0–300 watts (± 30 watts) in 1-watt increments. Pressure is programmable from 0 to 300 psi (0–21 bar). This instrument is equipped with an IR temperature sensor that sees through glass and Teflon[®] (iWave[®], Charlottetown, PE, Canada), and vent and resealing technology are used for the safe handling of over-pressurization (ActiVent, Auburn, CA, USA). Different experimental conditions were tested three different times at two power modes and three temperatures.

The solvent evaporation process was carried out as previously described during ASE. The solvent was separated from the solid via gravity filtration. The parameters that have the greatest influence on extraction via MAE were selected using a factorial design.

2.5. Suberin Determination and Desuberized Fraction

As explained below, suberin determination could not be carried out using the ASE system; the suberin determination of extractive-free cork was carried out according to the classical or traditional methodology [13]. The entirety of the sample, obtained after ASE using dichloromethane, ethanol, and water to remove extractives from cork, underwent reflux with 250 mL of a 3% NaOCH₃ solution in CH₃OH for 3 h. This was followed by the filtration of the liquid residue, and the solid residue was then subjected to a second reflux with CH₃OH for 15 min. After filtration, the merged liquid fractions were blended, acidified to pH 6 with 2 M H₂SO₄, and evaporated to near dryness using a rotating evaporator (Rotavapor Heidolph). This residue was suspended in 100 mL H₂O and extracted with 100 mL CHCl₃ three times. The resulting residue was dispersed in 100 mL H₂O and subjected to three extractions with 100 mL of CHCl₃. The combined extracts were dehydrated using Na₂SO₄, filtered, evaporated, and quantified gravimetrically as suberin. Polar suberin monomers, such as glycerol, were not extracted with chloroform, and they remained in the aqueous phase. The non-saponifiable fraction (the desuberized fraction) was weighed, and this fraction was considered as the remaining lignin and holocellulose content.

2.6. Data Analysis

Different full factorial designs were used sequentially to optimize ASE and MAE. The full factorials had two or three factors with two or three levels. The data processed with Microsoft Excel (Microsoft 365, update 2023) and Minitab program version 18 for Windows were used for data analysis.

3. Results

3.1. Experimental Design of ASE

Three full factorial designs (two or three levels) with four factors (temperature and time extraction, sample quantity, and the number of extractions) were used. A total of 36 experiments—12 points for the factorial design—were carried out according to a randomized order. In accordance with the boiling points of solvents (dichloromethane 40 °C [14], ethanol 78 °C [14], and water 100 °C [15]), the literature and the manufacturer's specifications, the following ASE was tested (Table 1). The response variables selected were the milligrams of extract obtained and the extraction ratio or yield as g extract/g raw cork·100. The suberin content was determined using the sample free of extractives. The first step for the determination of the suberin content was based on alkaline methanolysis (3 h), using a Soxhlet extractor with 3% sodium methoxide. The operating instructions with respect to the equipment do not recommend the use of a strong basic solution. For this reason, the use of alkaline methanolysis with the ASE methodology was discarded.

Table 1. ASE parameters used for the ASE study of the cork extractive optimization extraction.

Factors	Levels									
	Solvent	Dichloromethane			Ethanol			Water		
Temperatures (°C)		40	60	70	90	100	120			
Time (min)		2	10	30	2	10	30	2	10	30
Sample weight		5	8	5	8	5	8			

3.2. Optimizing Cork Extractive Chemical Composition via ASE

As mentioned in the previous section, three full factorial designs with four factors were used to optimize the dichloromethane, ethanol, and water extractives' extraction in cork. Next, the Pareto chart of the standardized effects, interaction plots, and main effect plots were evaluated, which take into consideration the influence of extraction yields for each solvent (Figure 3). Although the extractions were carried out in sequence, that is, the extraction in dichloromethane was first carried out, followed by the extraction in ethanol and finally the extraction in water, each phase/solvent was evaluated separately. In the dichloromethane, ethanol, and water extractions, the sample's quantity, temperature, and interaction times seemed to be determining factors in making the extraction more efficient. The analytical data showed that the highest temperatures and longest durations had the best extraction yield. However, the sample's quantity exhibited an inversely proportional relationship with respect to the extraction yield. These results permitted the fixing of the sample's quantity and the exploration of new temperatures and extraction times, resulting in new factorial designs. Extraction times from 30 min to 90 min were explored. Serial extractions for the same solvent were investigated in order to observe whether extraction yields increased when adding consecutive extractions with the same solvent (Figure 4). In the case of the dichloromethane extractions, the yield of the second extraction or cycle was very low (below 0.5%). However, in the case of the ethanol and water extractions, the yield for the second or third cycle was more than 1%. For this reason, the number of cycles were included in the optimization process. After the optimization analysis of the extracts obtained via ASE, the conditions that produced the best yield are shown in Table 2.

Table 2. The optimization parameters of cork extractive determination via ASE.

	Extractives in DCM	Extractives in Ethanol	Extractives in Water
Sample weight	5 g	5 g	5 g
Solvent	Dichloromethane	Ethanol	Distilled water
Cell size		100 mL	
Extraction temperature	40 °C	90 °C	100 °C
Static time	30 min	40–60 min	60–90 min
Number of cycles or extractions	1	1 or 2	2 or 3

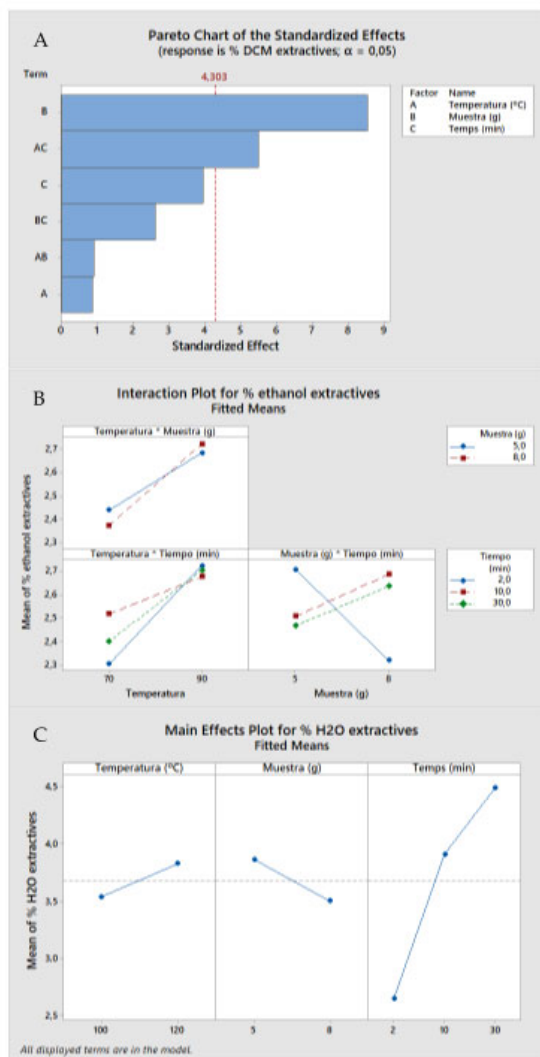


Figure 3. Optimization of the dichloromethane, ethanol, and water extractive extraction in cork: (A) Pareto chart of the standardized effects for the dichloromethane extractions, (B) the interaction plots for the ethanol extractions, and (C) the main effects plots for the water extractions (from the left to right).

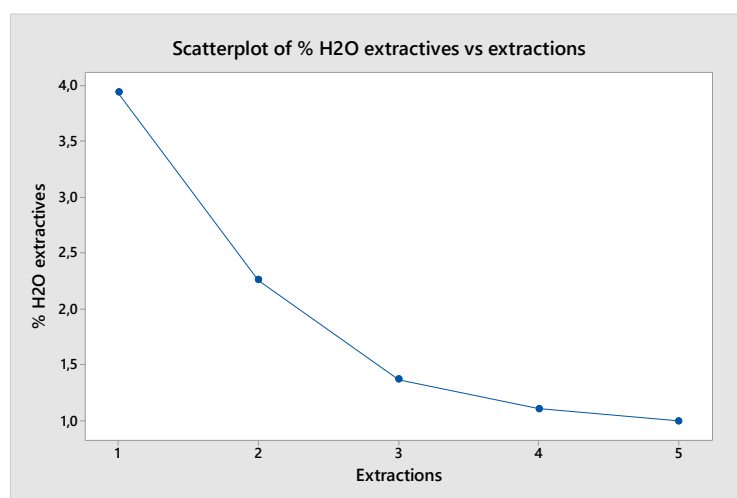


Figure 4. Serial extractions for the water extractions.

3.3. Validation of Cork Extractive Determination via ASE

Two trials were prepared for the analysis of the chemical composition of cork with respect to the classical method and for the ASE-optimized methodology (Table 3). A statistical *t*-test comparison of the two methodologies showed no significant differences (*p*-value > 0.05).

Table 3. Chemical composition of cork with different methodologies: traditional, ASE studied method (*n* = 4).

	% Extractives Dichloromethane	% Extractives in Ethanol	% Extractives in Distilled Water	% Subrin	% Lignin-Polysaccharides Ashes
Traditional method	4.82 ± 0.86	3.45 ± 0.82	4.16 ± 0.71	36.37 ± 6.56	51.36 ± 8.74
ASE studies method	3.62 ± 0.54	2.66 ± 0.46	6.17 ± 1.65	35.15 ± 4.03 *	52.39 ± 3.45 *

* The suberin content and the lignin-holocellulose-ash percentage were obtained by the traditional method as explained above.

3.4. Extractions via MAE

In accordance with the boiling points of solvents, the literature, and the manufacturer's specifications, the following microwave-assisted extractions (MAE) were tested with Discover SP[®] microwave synthesis reactors. In the case of the extraction process via MAE, the optimization process was not carried out, but the conditions in Table 4 were taken into account in accordance with the Discover team's technical assistance. Two trials were prepared for the analysis of the chemical composition of cork with respect to the classical method and MAE. The results obtained via both methodologies are shown in Table 5.

Table 4. MAE parameters used for the chemical composition of cork.

	Extractives in DCM	Extractives in Ethanol	Extractives in Water	Suberin Content
Extraction temperature	40 °C	70 °C	80 °C	80 °C
Solvent	dichloromethane	ethanol	distilled water	sodium methoxide solution 3%
Sample/solvent ratio	4%			
Mode	Open vessel *			
Power	100 W	80 W	20 W	100 W
Extraction time	20 min	10 min	30 min	10 min

* Open vessel with 100 mL round bottom flask.

Table 5. Chemical composition of cork with different methodologies: traditional, MAE studied method (*n* = 4).

	% Extractives Dichloromethane	% Extractives in Ethanol	% Extractives in Distilled Water	% Subrin	% Lignin-Polysaccharides-Ashes
Traditional method	5.42 ± 0.21	4.15 ± 1.15	9.70 ± 2.02	46.39 ± 6.56	34.33 ± 8.74
MAE studies method	2.85 ± 1.36	2.07 ± 0.36	1.64 ± 0.42	45.11 ± 2.62	48.33 ± 1.32 *

* The lignin-holocellulose-ash percentage was obtained by the traditional method as explained above.

4. Discussion

The characteristics of a material are influenced by the chemical traits of its constituents, their proportions, and their distribution within the solid structure. In cork, similarly to other cellular materials, the chemical elements are situated in the cell faces and edges [3]. Cork's composition differs from that of other plant tissues. It is believed that cork prevents water loss, regulates gas transfer, and hinders the passage of large molecules and microorganisms. Chemically, cork is primarily composed of suberin as the main structural cell

wall component, and it is closely associated with other elements, mainly lignin and non-lignin aromatics. This complexity poses challenges in fully comprehending cork's chemical composition. The conventional approach to chemically characterizing cork, similarly to other lignocellulosic materials, involves reporting its cumulative chemical composition, including inorganic ashes, organic extractives, and structural components (suberin, lignin, and polysaccharides). Pereira H. [3] emphasizes that the specific chemical protocol can directly impact the results, underscoring the need for meticulous data analyses, especially for comparative purposes. While there have been some proposals for the chemical fractionation of cork, the most widely adopted and robust approach has evolved by adapting standard methodologies for lignocellulosic materials and incorporating suberin removal and determination [13]. Currently, the approach proposed by Marques and Pereira in 1987 is accepted and used by scientists who study cork compositions.

Although the proposal from the late 1980s is still used, some methodological modifications have been made to optimize times or select target compounds [6,7,16]. The present study is an example of this: the protocol that defines a sequential gravimetric assay and the solvents used are maintained, but the extraction technique is optimized, moving from using a Soxhlet extractor to a new, more efficient technique. Currently, new technologies have emerged, and they may constitute a potential tool to reduce solvent amounts, treatment times, and temperatures, as well as energy consumption, thus improving the recovery of valuable compounds with lower costs [17,18]. MAE and ASE have been studied as alternatives to the Soxhlet methodology used for the determination of extractives in dichloromethane, ethanol, and water, which are the first steps in the analysis of the chemical composition of cork [9].

According to the results, ASE and the traditional methodologies did not present statistical differences and could be considered equivalent. Moreover, the chemical composition results obtained via ASE (12% of extractives, 35% suberin, and 52% lignin and polysaccharides) are within the ranges described in the bibliography (14–18% of extractives, 50% suberin, and 40–45% lignin and polysaccharides) [3,4]. ASE could be considered a sustainable solution to the traditional methodology for analyzing the chemical composition of cork for two reasons: the testing time was reduced by 3 times, and the amount of solvent was reduced by half.

The percentages of the extractives obtained via MAE are three times lower (6.56% total amount) than the percentages obtained via the classical method (19.27% total amount). However, the percentages of suberin are very similar in both methods: 45.11% and 46.39%, respectively. The results of the chemical composition of cork obtained via MAE suggest that it could be an applicable methodology for this analysis. However, it is necessary to carry out more research and optimization processes in this field.

5. Conclusions

Chemically, the composition of cork has been adapted from wood and other lignocellulosic analytical methods. This analysis is based on a sequential gravimetric extraction of the different components of cork. The experimentation time (approx. 40 h) is long and the amount of required organic solvents is significant (1.2 L) for each sample, making its implementation as a routine quality test in cork manufacturing difficult. In this study, two approaches were presented as alternatives to the classical methodology for determining the chemical composition of cork: ASE and MAE. According to the results, the determination of cork extractives via ASE was optimized and validated. No significant differences were found between the content of extractives in the dichloromethane, ethanol, and water obtained via ASE compared to the results obtained with the classical methodology. Moreover, ASE could be considered a sustainable solution to the traditional methodology for analyzing the chemical composition of cork for two reasons: the testing time was reduced by 3 times, and the amount of solvent was reduced by half.

On the other hand, the MAE methodology for the analysis of the chemical composition of cork was developed. The MAE methodology needs to be optimized in the future. However, MAE and ASE are quicker methods, use less solvent, and provide more reproducible results than the classical chemical composition methodology. These data pave the way for novel sustainable alternatives with respect to the studies of the chemical composition of cork.

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References

1. Rives, J.; Fernandez-Rodriguez, I.; Gabarrell, X.; Rieradevall, J. Environmental analysis of cork granulate production in Catalonia—Northern Spain. *Resour. Conserv. Recycl.* **2012**, *58*, 132–142. [[CrossRef](#)]
2. Pereira, H.; Emília Rosa, M.; Fortes, M.A. The cellular structure of cork from *Quercus suber* L. *IAWA J.* **1987**, *8*, 213–218. [[CrossRef](#)]
3. Pereira, H. *Cork: Biology, Production and Uses*, 1st ed.; Elsevier: Oxford, UK, 2011.
4. Silva, S.P.; Sabino, M.A.; Fernandes, E.M.; Correló, V.M.; Boesel, L.F.; Reis, R.L. Cork: Properties, capabilities and applications. *Int. Mater. Rev.* **2005**, *50*, 345–365. [[CrossRef](#)]
5. Jové, P.; Olivella, M.À.; Cano, L. Study of the variability in chemical composition of bark layers of *Quercus suber* L. from different production areas. *BioResources* **2011**, *6*, 1806–1815. [[CrossRef](#)]
6. Conde, E.; Cadahía, E.; Garcia-Vallejo, M.C.; González-Adrados, J.R. Chemical characterization of reproduction cork from Spanish *Quercus Suber*. *J. Wood Chem. Technol.* **1998**, *18*, 447–469. [[CrossRef](#)]
7. Pereira, H. Composição química da raspa em pranchas de cortiça de reprodução amadia. *Bol. Inst. Prod. Florestais-Cortiça* **1987**, *587*, 231–233.
8. Wise, L.E. Chlorite holocellulose, its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses. *Tech. Assoc. Pap.* **1946**, *29*, 210–218.
9. Bouras, M.; Chadni, M.; Barba, F.J.; Grimi, N.; Bals, O.; Vorobiev, E. Optimization of microwave-assisted extraction of polyphenols from *Quercus* bark. *Ind. Crops Prod.* **2015**, *77*, 590–601. [[CrossRef](#)]
10. Qiu, C.; Wang, A.; Ullah, R.; Liu, Y.; Wang, M.; Lu, D.; Galbiati, F. *Determination of Polycyclic Aromatic Hydrocarbons in Soils Using the EXTREVA ASE Accelerated Solvent Extractor and GC-MS*; Application Note 001106; Thermo Fisher Scientific: Sunnyvale, CA, USA; Thermo Fisher Scientific, China Innovation Center: Shanghai, China, 2022.
11. Alahmad, W.; Kaya, S.I.; Cetinkaya, A.; Varanusupakul, P.; Ozkan, S.A. Green chemistry methods for food analysis: Overview of sample preparation and determination. *Adv. Sample Prep.* **2023**, *5*, 100053. [[CrossRef](#)]
12. Herrero, M.; Martín-Álvarez, P.J.; Senorans, F.J.; Cifuentes, A.; Ibáñez, E. Optimization of accelerated solvent extraction of antioxidants from spirulina platensis microalga. *Food Chem.* **2005**, *93*, 417–423. [[CrossRef](#)]
13. Marques, A.V.; Pereira, H. On the determination of suberin and other structural components in cork from *Quercus suber* L. *An. Inst. Super. Agron.* **1987**, *42*, 321–335.
14. O’Neil, M.J. (Ed.) *The Merck Index—An Encyclopedia of Chemicals, Drugs, and Biologicals*; Royal Society of Chemistry: Cambridge, UK, 2013; p. 1124.
15. Haynes, W.M. (Ed.) *CRC Handbook of Chemistry and Physics*, 95th ed.; CRC Press LLC: Boca Raton, FL, USA, 2014; pp. 98, 246.
16. Marques, A.V.; Pereira, H.; Meier, D.; Faix, O. Isolation and characterization of a guaiacyl lignin from saponified cork of *Quercus suber* L. *J. Holzforsch.* **1996**, *50*, 393–400. [[CrossRef](#)]

17. Barba, F.J.; Esteve, M.J.; Tedeschi, P.; Brandolini, V.; Frígola, A. A comparative study of the analysis of antioxidant activities of liquid foods employing spectrophotometric, fluorometric, and chemiluminescent methods. *Food Anal. Methods* **2013**, *6*, 317–327. [[CrossRef](#)]
18. Deng, Q.; Zinoviadou, K.G.; Galanakis, C.M.; Orlie, V.; Grimi, N.; Vorobiev, E.; Lebovka, N.; Barba, F.J. The effects of conventional and non-conventional processing on glucosinolates and its derived forms, isothiocyanates: Extraction, degradation, and applications. *Food Eng. Rev.* **2014**, *7*, 357–381. [[CrossRef](#)]

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