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From Waste to Resource: Compositional Analysis of Olive Cake's Fatty Acids, Nutrients and Antinutrients

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Abstract: The olive oil industry, recognised for its beneficial products for health and food culture, generates a significant amount of by-products that, if not appropriately managed, can pose considerable environmental challenges. This study examined six olive cakes (OC) from the Trás-os-Montes and Alto Douro regions, collected on different dates and mills: two obtained by pressing (COC), two by centrifugation (TPOC), including one partially pitted and one dehydrated, and two exhausted (EOC), which were subjected to conventional chemical analyses, namely dry matter (DM), organic matter (OM), crude fat (CF), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) profiling fatty acid (FA) and phosphorus and phytic acid content. The dehydrated TPOC had only 8% moisture content (due to drying), followed by EOC with 10% and COC (65–79%). The CF content was high in COC 1 (14.5% in DM), residual in EOC (1.5%) and intermediate in TPOC (9–10%). CP ranged from 5.3 to 7.3%. Notably, NDF levels were high (>65% in 5 samples; pitted TPOC 57.4%) and very lignified (ADL > 23%). Different FA profiles were observed: COC had the highest monounsaturated (76.36 g/100 g), while EOC had the highest saturated (16.56 g/100 g) and polyunsaturated (14.14 g/100 g). Phosphorus and phytic acid content (g/100 g) of EOC 2, TPOC pitted, TPOC dehydrated, COC 1 and COC 2 showed similar values to each other (mean of 0.12 ± 0.02 and 0.44 ± 0.0, respectively), with EOC 1 having the lowest levels (0.07 ± 0.01 and 0.26 ± 0.04, respectively). These results highlight the potential of OCs, especially dry TPOC, which offers transport, conservation and utilisation benefits.

Keywords: exhausted olive cake; phytic acid; nutritional profile; waste management; circular economy

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1. Introduction

The olive tree, scientifically known as *Olea europaea* L., is a small tree that belongs to the *Oleaceae* family. It is mainly found in the Mediterranean climate but is also commonly planted in tropical and subtropical regions worldwide. Countries such as Greece, Italy, Spain, Australia, Portugal, France, Cyprus, Israel, Jordan, the US, Morocco, Turkey and Tunisia are among the leading olive producers [1,2]. Although its cultivation has expanded to various corners of the globe, the Mediterranean remains the main centre, covering approximately 98% of the world's olive production, with Spain, Italy and Greece standing out [1,3]. As a result, the European Union (EU) accounts for around 70% of global olive production, generating a production value of approximately EUR 7000 million annually.

The olive tree is renowned for its fruit, widely used in producing olive oil, making it a valuable cash crop in these countries. This, in turn, makes the olive industry a critical factor in developing the agro-industrial sector and a social and economic driver for the EU's southern regions [4,5].

In 2023, there were 104 olive oil mills in Trás-os-Montes and Alto Douro (23% of the mills in Portugal) (National Institute of Statistics). Olive oil production in the 2019–2020 campaigns at Portuguese mills is estimated to have reached 140,500 tons, an all-time high (SIAZ, 2020).

During the extraction process, 1000 kg of olives produces about 800 kg of waste [6]. Olive oil extraction involves several stages, such as olive washing, grinding, beating, and extraction, which is the fundamental step [7]. However, improper disposal of this by-product can represent a potential environmental problem [8].

Among the stages in the production of extra virgin olive oil (EVOO), the extraction phase has plenty of room for improvement to increase yield, product quality and the environmental burden of the entire production cycle [9]. In the traditional separation process, the resulting paste is subjected to pressure between two mats, resulting in the solid part and a liquid fraction of oil and water, known as pomace or crude olive cake. This by-product consists mainly of seed oil and is, therefore, the richest in energy (around 10.4% EE) [10–12]. Over time, this liquid fraction can be separated into two distinct parts. However, in recent decades, the olive oil industry has taken over centrifugation, offering three-phase and two-phase systems (Figure 1) [7,13]. The fundamental difference between the two systems lies in the presence or absence of added water during extraction. In the three-phase system, a significant amount of water (usually around 50%) is added to the paste of crushed olives before centrifugation, generating three fractions at the end of the process: solid (olive skin or olive cake) and two liquids (oil and wastewater) [7,14]. This has been the main drawback of this extraction system since these waters are highly polluting [15]. In the two-phase system, no water is added during the extraction process, which generates two distinct phases: oil and olive cake. Despite being labelled as an ecological system due to the significant reduction in the generation of wastewater and its contaminant load, it still generates very wet solid waste as a by-product [14–16].

Some mills still carry out the procedure of dehydrating the resulting olive cake followed by extraction of the remaining oil (which represents around 2% of its weight) using a solvent. Hexane is the solvent usually used for this industrial solid-to-liquid extraction and the resulting by-product, exhausted olive cake (EOC), contains smaller fragments of pulp, skins, seeds and stones, retaining around 10% moisture content [17–19].

The chemical composition of olive cake can vary based on the climate and extraction method used [20]. Its composition includes the stone (18–32%), pulp, shell, kernel, residual oil and approximately 40–60% water. The main elements present in solid olive by-products are natural polymers, namely lignin (26–30%), hemicellulose (7–9%) and cellulose (7–9%), proteins (5–7%), fatty acids (5–8%) and phenolic compounds (oleuropein, hydroxytyrosol and tyrosol) [21]. This chemical structure highlights the solid waste from olive processing as a promising source of bioactive compounds and nutrients, especially in light of the growing search for natural alternatives to synthetic ingredients [11,16,20,22]. Efficient extraction methods can maximise the utilisation of these compounds, contributing to sustainability and added value in the olive oil industry. Here are several methods for extracting components from olive cake [23]: (1) Solvent extraction: This method involves using solvents to dissolve and extract specific compounds from the olive cake. Common solvents include ethanol, methanol, hexane and water. Solvent extraction is widely used due to its efficiency and ability to extract a wide range of compounds; however, the choice of solvent is critical as it affects the yield and purity of the extracted compounds. Additionally, solvent recovery and disposal can pose environmental challenges [24]; (2) Supercritical fluid extraction (SFE): This method uses supercritical fluids, typically carbon dioxide (CO_2), at high pressure and temperature to extract components. SFE is a green technology that avoids the use of harmful organic solvents. It is highly selective and can produce high-purity extracts. The

disadvantages of this method are associated with the initial setup cost for SFE equipment, which is high, and the fact that the process requires precise control of temperature and pressure [25]; (3) Ultrasound-assisted extraction (UAE): UAE uses ultrasonic waves to create cavitation bubbles in the extraction solvent, which helps to break down cell walls and enhance the release of intracellular compounds. This method is relatively quick, energy efficient and can be conducted at lower temperatures, preserving the integrity of heat-sensitive compounds. The efficiency of UAE can be influenced by the ultrasonic frequency and power as well as the properties of the solvent and the sample [26]; (4) Microwave-assisted extraction (MAE): This method uses microwave energy to heat the sample and solvent, which accelerates the extraction process. MAE is fast, efficient and can reduce solvent usage. It is particularly effective for extracting polar compounds. The method may not be suitable for nonpolar compounds and there is a risk of overheating or degradation of sensitive compounds [26]; (5) Enzyme-assisted extraction (EAE): This method employs specific enzymes to break down cell walls and release the desired compounds from the olive cake. This method can enhance the yield and selectivity of the extraction process. It is environmentally friendly and can be conducted under mild conditions. The cost of enzymes can be high and the process parameters need to be carefully optimised to achieve the best results [27]; (6) Pressurised liquid extraction (PLE): Also known as accelerated solvent extraction, PLE uses high pressure and temperature to enhance the extraction efficiency of solvents. PLE is faster than traditional solvent extraction and can use smaller amounts of solvent. It is effective for extracting both polar and nonpolar compounds; however, the equipment cost is relatively high and there is a risk of thermal degradation of heat-sensitive compounds [28].

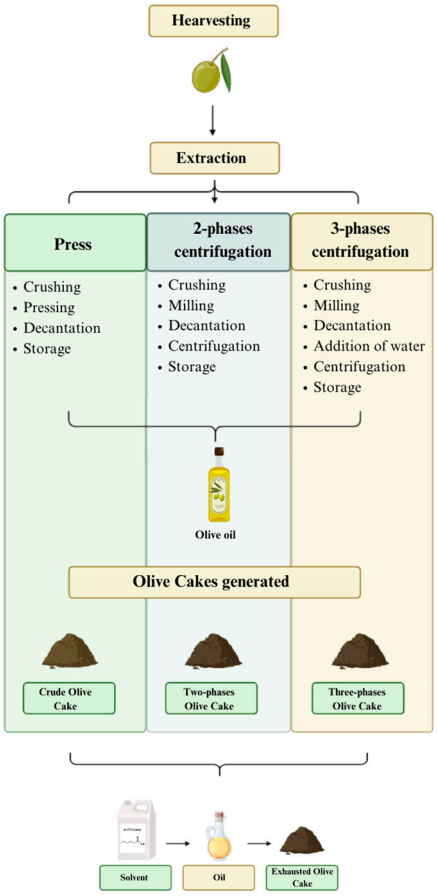

Figure 1. An overview of olive oil extraction, emphasising the different by-products generated by each extraction method.

Selecting the appropriate extraction method for olive cake components depends on several factors, including the nature of the target compounds, environmental considerations and economic viability. Combining different methods or optimising existing ones can lead to improved yields and purities, contributing to the sustainable and efficient use of olive cake by-products in various industries.

The by-product in question, which is still little exploited, generates disposal costs. The most common options are burning it or burying it in the ground along with other waste and, in less frequent cases, it is used as a supplement for animal feed [4,29]. However, some valuable compounds can be recovered through the by-products of olive oil extraction, namely specific polyphenols, proteins, fats, cellulose and lignin. These by-products can also be converted into new, higher-value-added products such as bioenergy, bio fertilisers, purified water, biobased materials and food and feed additives [30]. Phenolic compounds are especially attractive for cosmetic and biomedical applications due to their antioxidising, anticarcinogenic and anti-inflammatory properties [10,31,32]. In this way, the bioeconomy has emerged as a promising model for agriculture. The European Commission (2012) defines it as the transformation of renewable biological resources into high-value-added products, from food and feed to bioproducts and bioenergy [33]. In addition, the European Catalogue of Feed Materials defines "ex-food" or "FFPs" as food products that do not originate from catering waste and that have been manufactured in full compliance with European food legislation [33,34]. This approach opens up a range of opportunities for the sustainable development of agriculture, valorising by-products and waste, boosting innovation, and diversifying production [35]. This study aimed to conduct a thorough analysis of the chemical composition of diverse olive cakes, shedding light on their fatty acid profile while also assessing their phosphorus and phytic acid content to explore potential applications in industries such as food, cosmetics, pharmaceuticals, and agriculture.

2. Materials and Methods

2.1. Samples of Olive Cakes

In the present study, six olive cakes (OC) were collected on different dates and mills in the region of Trás-os-Montes and Alto Douro, Portugal: two obtained by pressing (COC), two by two-phase centrifugation, including one partially pitted, one dehydrated (TPOC), and two exhausted (EOC), Table 1. Representative samples of each type of olive cake were collected during the 2020/21 olive oil campaigns and the olive cakes were frozen at −20 °C until further analysis.

Table 1. Origin of olive cakes.

Olive Cakes	Oil Mill	Campaigns
EOC 1	Quinta das Covas	jan/21
EOC 2	Gimonde	mai/21
TPOC (pitted)	Murça	mai/21
TPOC (dehydrated)	Frechas	dez/21
COC 1	Baçal	mai/21
COC 2	Baçal	dez/21

EOC: Exhausted olive cake; TPOC: two-phase olive cake; COC: crude olive cake.

2.2. Chemical Composition of Olive Cakes

The samples were dried in a forced-air oven at 50°. They were then crushed in a 1 mm sieve to homogenise and prepare for chemical analysis. The levels of dry matter (DM), organic matter (OM) and crude fat (CF) of the olive cake were determined according to the methodologies proposed by the Association of Official Analytical Chemists (AOAC) [36]. The crude protein (CP) content was calculated by multiplying its nitrogen content by a factor of 6.25. The EE content was determined by extracting the samples in a Soxlet apparatus using petroleum ether as a solvent. The methodologies proposed by Robertson

and Van Soest [37] were used to determine the fractions of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL).

2.3. Fatty Acid Profile

The fatty acid profile of olive cakes and the experimental diet samples was measured by gas chromatography and the fatty acid methyl esters (FAME) were analysed in an Agilent Technologies Spain GC-Agilent 6890N gas chromatograph (Madrid, Spain) equipped with a flame ionisation detector and an HP 7683 automatic sample injector. The fatty acid profile was determined as the proportion of saturated, monounsaturated and polyunsaturated fatty acids (SFA, MUFA and PUFA, respectively) in grams per 100 g of fatty acids (%), and was then determined according to ISO 12966-2:2011 [38].

2.4. Phosphorus and Phytic Acid Content

To determine the phytic acid content in the samples, we followed the protocol provided by the Megazyme commercial kit (K-PHYT) [39]. The samples were subjected to an acid extraction followed by treatment with phytase and phosphatase to release the phosphorus. This method was carried out in triplicate for each sample and the total phosphate content released was measured using a colourimetric reaction with ammonium molybdate. The amount of molybdenum blue formed in this reaction is directly related to the amount of inorganic phosphate (Pi) present in the sample and is quantified by the increase in absorbance at 655 nm (Figure 2). Pi is determined as phosphorus using a calibration curve generated with standards of known phosphorus concentration (0.0, 0.5, 2.5, 5.0 and 7.5 mg/mL in distilled water) and 1 mL of each sample was applied to the phytic acid assay in triplicate (n = 3). The results were expressed in g of phosphorus per 100 g sample (g P/100 g).

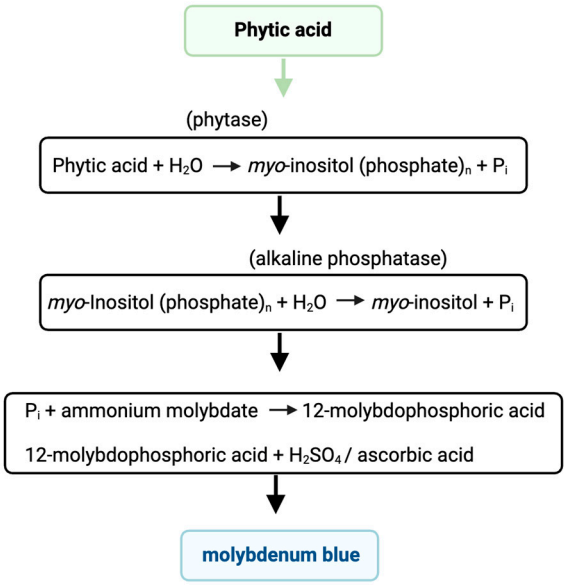

Figure 2. Biochemical reactions involved in the degradation of phytic acid.

2.5. Statistical Analysis

The regression equation (y = ax ± b), coefficient of determination (R^2), adjusted R^2 and correlation coefficient (r) were also calculated for each method. The data obtained were checked for normality using the Shapiro–Wilk's test and subjected to variance analysis (ANOVA) and a multiple range test (Tukey's test/t student test) for a p-value < 0.05 using JMP statistics 17.1.0 software (JMP, Cary, NC, USA). All sample results were presented as mean values ± standard deviation.

3. Results and Discussion

3.1. Chemical Composition of Olive Cakes

Table 2 presents the chemical composition of different olive cakes, expressed as a percentage of dry matter (DM). Several parameters are evaluated, including OM, NDF, ADF, ADL, cellulose (CEL), CP and crude fat (CF). Observing the data, it is evident that the composition varies significantly among the different types of olive cakes. For instance, the dehydrated TPOC and COC 1 exhibit the highest DM content, surpassing 90%, indicating a relatively low moisture content. On the other hand, TPOC (pitted) has the lowest DM content, indicating a higher moisture content compared to the other samples. Regarding OM, COC 2 shows the highest value, suggesting a higher concentration of organic matter compared to other samples. This could be attributed to factors such as the olive variety and processing method. NDF and ADF are indicators of the fibre content in the olive cakes. Generally, TPOC (dehydrated) shows the highest NDF content, while EOC 2 displays the highest ADF content. These differences could be due to variations in the olive fruit composition and processing techniques. ADL and CEL are components of fibre associated with the structural integrity of plant cell walls. EOC 2 and COC 2 exhibit the highest ADL and CEL content, indicating differences in the lignin and cellulose composition among the samples. CP content reflects the protein content in the olive cakes. Notably, EOC 2 and COC 2 show the highest CP content, suggesting a higher protein concentration compared to other samples. Finally, CF represents the ether extract content, which includes lipids and other organic compounds. COC 1 has the highest CF content, followed by EOC 1, indicating a higher lipid concentration in these samples compared to others. Overall, the data illustrate the diverse chemical composition of olive cakes, influenced by various factors such as olive variety, processing methods and environmental conditions. Understanding these differences is crucial for potential applications in industries such as agriculture, food and bioenergy.

Table 2. Chemical composition of the olive cakes (g/100 g).

Chemical Composition (% DM)	Olive Cakes					
	EOC 1	EOC 2	TPOC (Pitted)	TPOC (Dehydrated)	COC 1	COC 2
DM	90.16 ± 0.03^{b}	89.79 ± 0.03^{b}	35.41 ± 0.74^{e}	96.76 ± 0.54^{a}	69.62 ± 0.30^{c}	65.53 ± 0.47^{d}
OM	94.15 ± 0.01^{d}	93.54 ± 0.01^{e}	91.20 ± 0.00^{f}	97.61 ± 0.00^{b}	96.91 ± 0.10^{c}	98.42 ± 0.02^{a}
NDF	67.97 ± 0.16^{b}	65.09 ± 0.92^{c}	57.43 ± 0.02^{d}	75.72 ± 0.98^{a}	67.68 ± 0.27^{b}	77.98 ± 0.25^{a}
ADF	36.77 ± 1.26^{c}	52.36 ± 0.51^{b}	51.18 ± 0.3^{b}	54.31 ± 1.57^{ab}	52.27 ± 0.27^{b}	57.68 ± 0.14^{a}
ADL	24.19 ± 0.01^{bc}	25.63 ± 0.23^{ab}	23.32 ± 0.97^{c}	25.87 ± 0.16^{a}	26.16 ± 0.09^{a}	26.53 ± 0.04^{a}
CEL	25.67 ± 0.17^{b}	26.74 ± 0.28^{b}	27.87 ± 0.67^{b}	28.45 ± 1.72^{ab}	26.11 ± 0.18^{b}	31.15 ± 0.17^{a}
CP	6.84 ± 0.72^{a}	7.26 ± 0.36^{a}	6.75 ± 0.11^{a}	6.33 ± 0.08^{ab}	5.38 ± 0.02^{b}	6.50 ± 0.13^{ab}
CF	1.51 ± 0.05^{d}	1.45 ± 0.04^{d}	10.03 ± 0.06^{b}	9.14 ± 0.08^{bc}	14.5 ± 0.59^{a}	8.43 ± 0.43^{c}

EOC: Exhausted olive cake; TPOC: two-phase olive cake; COC: crude olive cake. DM: dry matter; OM: organic matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; CEL: cellulose; CP: crude protein; CF: crude fat. Mean ± standard deviation of determinations ($n = 2$). Different letters in the same column correspond to significant differences between species ($p < 0.05$). ANOVA followed by a post hoc Tukey test.

The chemical composition of olive cake can vary according to the climate and the extraction method used. These factors directly impact its constituents—pericarp, mesocarp, endocarp and stone each with distinct chemical compositions. Notably, olive cake is distinguished by its high fibre content, often highly lignified, and significant protein content, typically around 10% [8,40].

Consistent with previous studies, our findings align with reported chemical composition values [41,42]. Notably, there is a significant difference in moisture content ($p < 0.001$) between TPOC pitted (64.59 ± 0.074) and TPOC dehydrated (3.24 ± 0.54), attributable to the drying process applied. Traditional or three-stage mills yield olive cakes with approxi-

mately 25% moisture content, while two-phase mills tend to produce cakes with higher water content due to the inclusion of the entire aqueous phase from the olive [41]. Comparing olive cake composition across studies proves challenging due to diverse influencing factors. These include olive origin, fruit ripeness, agronomic practices, soil and climate conditions during cultivation, extraction processes and oil storage conditions. Despite these challenges, comprehending an olive cake's variable composition is crucial for optimising its utilisation across various industries [43–46].

As expected, the CF content was significantly lower in the EOCs ($p < 0.001$). This is due to the fact that, in mills, this olive cake is commonly subjected to a final extraction process with a solvent (hexane) to remove the oil from the cake [47,48]. COC had the highest CF content (mean of 14.40 ± 0.51). This olive cake is obtained by pressing in traditional mills and stands out for having an intermediate dry matter content, low levels of crude protein and a high fat content [49–51]. The CF content in the COC (8.43–14.5 g/100 g) is in line with the literature for this extraction system, demonstrating consistency with the literature, where other authors have found values ranging from 3.3 to 16.2 g/100 g of CF for cakes from the same extraction system [52–54]. On the other hand, the results obtained for olive cakes from the two-phase extraction system (TPOCs) showed values ranging from 9.0 to 10.0%, in contrast to the findings of other authors, who reported content of 13% for olive cakes from the same extraction system [48]. Distinguishing between the three-phase and two-phase extraction systems is fundamental. The main disparity lies in a higher moisture content and lower oil content in the two-phase extraction system, reflecting more efficient and ecologically sustainable centrifugation than the three-phase system, resulting in process efficiency and lower environmental impact [52]. The fibre fraction in olive cakes consistently constitutes the highest proportion (44–58%) compared to other components [41]. Specifically, NDF contents were highest in COC 2 and TPOC (dehydrated), with values ranging from 57 to 58% (mean of 76.85 ± 0.61; $p < 0.001$), while TPOC pitted exhibited the lowest content at 57.43 ± 0.03. This notable fibre abundance aligns with findings from various studies investigating olive cake composition [8,50,53–55].

The lignin content remained consistent across all the cakes, with COC 2 exhibiting the highest content (26.53 ± 0.04) and TPOC (pitted) the lowest (23.32 ± 0.97). Regarding cellulose, values were statistically similar across all cakes (mean of 26.96 ± 0.61, $p < 0.03$), except for COC 2, which had the highest content (31.15 ± 0.17). These findings are in line with previous studies exploring similar variations of this by-product [56]. Olive cakes typically contain approximately 10% hemicellulose, 14–26% cellulose and 27% lignin, characteristics that classify it as a highly lignified by-product [56,57].

The crude protein content remained statistically similar across the samples (mean of 6.64 ± 0.28), with EOC 2 exhibiting the highest content (7.26 ± 0.36) and COC 1 the lowest (5.38 ± 0.02), subject to variation based on the extraction method, with some samples recording levels as high as 11.3% [51]. Previous studies have reported similar values for EOC (7.2%) and higher ones for TPOC (pitted; 8.8%) and COC (7.6%) [56].

3.2. Phosphorus and Phytic Acid Content

The phosphorus and phytic acid content of the olive cakes analysed is listed in Table 3. The table presents the levels of total phosphorus and phytic acid in different types of olive cakes. Total phosphorus refers to the overall content of phosphorus in the samples, while phytic acid represents a specific form of phosphorus found in plant-based foods. Observing the data, it is evident that there are variations in both total phosphorus and phytic acid levels among the different types of olive cakes. For total phosphorus, EOC 2 displays the highest concentration, followed by TPOC (pitted) and TPOC (dehydrated). COC 1 and COC 2 exhibit similar levels of total phosphorus, while EOC 1 has the lowest concentration. These differences could be attributed to factors such as olive variety, soil composition and agricultural practices. Phytic acid levels also vary among the samples, with EOC 2 showing the highest concentration, followed by COC 1 and COC 2. TPOC (pitted) and TPOC (dehydrated) exhibit intermediate levels of phytic acid, while EOC 1 has the lowest

concentration. Phytic acid is known to be an antinutrient that can inhibit the absorption of minerals such as iron and zinc in the body. Therefore, higher levels of phytic acid in certain olive cakes could have implications for their nutritional value and bioavailability of minerals. Overall, the data highlight the importance of considering both total phosphorus and phytic acid levels in olive cakes as they can impact nutritional quality and potential applications in various industries such as food and agriculture. Further research is needed to understand the factors influencing phosphorus and phytic acid levels in olive cakes and their implications for human health and the environment.

Table 3. Phosphorus and phytic acid content of olive cakes (g/100 g).

	Olive Cakes					
	EOC 1	**EOC 2**	**TPOC (Pitted)**	**TPOC (Dehydrated)**	**COC 1**	**COC 2**
Total Phosphorus	0.07 ± 0.01^{b}	0.14 ± 0.02^{a}	0.13 ± 0.02^{a}	0.11 ± 0.02^{ab}	0.12 ± 0.01^{a}	0.13 ± 0.01^{a}
Phytic Acid	0.26 ± 0.04^{b}	0.48 ± 0.06^{a}	0.45 ± 0.08^{a}	0.38 ± 0.07^{ab}	0.43 ± 0.02^{a}	0.46 ± 0.04^{a}

EOC: Exhausted olive cake; TPOC: two-phase olive cake; COC: crude olive cake. Mean ± standard deviation of determinations (n = 3). Different letters in the same column correspond to significant differences between species ($p < 0.05$). ANOVA followed by a post hoc Tukey test.

Phosphorus and phytic acid play crucial roles in plant materials, bearing significant nutritional and processing implications within olive cake. Phytic acid serves as the primary form of phosphorus storage in seeds and fruit, although it is less common in leaves. Nevertheless, when accurately measured, it is almost always detectable, constituting approximately 7.6% of total phosphorus [58]. During growth, seeds accumulate phosphorus beyond cellular requirements, which plants then convert into phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate or InsP6) [59]. In mature seeds, phytic acid stores roughly 60–80% of total phosphorus. However, its complex structure renders it unavailable for direct absorption by humans and nonruminant animals. Since many of these organisms lack phytase, the enzyme required to degrade phytic acid and release phosphorus, mineral deficiencies may occur. Additionally, phytic acid acts as a potent chelator of Fe, Mg and Ca, further impacting mineral absorption [60–62].

Data regarding the presence of phytic acid in the olive oil industry by-products and olive oil itself have yet to be consolidated and critically analysed comprehensively. In their examination of the literature on phytic acid content in leaves, Alkarawi and Zotz identified 45 published studies in which phytic acid was consistently detected whenever specifically investigated, constituting up to 98% of total phosphorus [61].

The absence of a commercially feasible, straightforward and quantitative method for phytic acid analysis has posed a significant hurdle to its accurate quantification. Although the widely accepted AOAC 986.11 method is valuable, it comes with limitations that impede its universal application. One major drawback is the necessity for a complex anion exchange purification step for each analysis. This step can be time-consuming, labour-intensive, error-prone and generates chemical waste. Another critical concern is the assumption made by this method that only phytic acid is purified during the anion exchange step. While this may hold true for unprocessed grains, where phytic acid constitutes the majority of inositol phosphates (at least 97%), it is not valid for processed foods and feeds. These materials may contain higher levels of smaller forms of myo-inositol phosphate (such as IP3, IP4 and IP5), which can coelute with phytic acid during purification, leading to an overestimation of the actual phytic acid content. Extreme phytic acid values, comparable to those found in plant storage organs, are often questionable and may be indicative of measurement errors, underscoring the need for more accurate and reliable methods [58,63–66].

EOC 2 exhibited the highest phosphorus (P) and phytic acid content, measuring 0.14 g/100 g and 0.48 g/100 g, respectively. Our phosphorus findings are consistent with those reported by others, who recorded values of 0.069 g/100 g P for olive cakes from the two-phase system and 0.12 g/100 g P for olive cakes from the three-phase system [20].

Studies analysing the phosphorus and phytic acid content of olives have documented values around 0.02% P and 0.12% phytic acid [67].

3.3. Fatty Acid Profile

Table 4 presents the fatty acid profile of various olive cakes, with the composition expressed as a percentage of the total fatty acids. Fatty acids are classified based on carbon chain length and degree of saturation. Upon examining the data, several trends and differences among the different types of olive cakes are notable. The comprehensive profile for each sample includes saturated fatty acids (SFA; C10:0, C14:0, C16:0, C18:0, C20:0, C22:0 and C24:0), with EOC 2 and EOC 1 exhibiting the highest levels (mean of 16.44 ± 0.00) and COC 1 the lowest levels (15.50 ± 0.00), monounsaturated fatty acids (MUFA; C16:1n-7, C18:1n-9, C18:1n-7, C20:1n-9 and C22:1n-9), with COC 1 and COC 2 showing the highest concentration (mean of 75.54 ± 0.00), followed closely by TPOC (pitted) (71.00 ± 0.00), EOC 1 (70.98 ± 0.05) and EOC 2 (69.30 ± 0.00) and finally polyunsaturated fatty acids (PUFA; C18:2n-6 and C22:1n-9), with EOC 2 having the highest levels of PUFA (14.14 ± 0.00), while COC 1 has the lowest levels (8.13 ± 0.00). This pattern is consistent with the typical fatty acid profile of olive oil, known for its high content of MUFA. Overall, these variations may be attributed to differences in the crude fat content of the sample, processing methods, olive cultivar varieties or even the storage form of the olive cakes. Dehydrated TPOC and COC 2 have the highest PUFA/SFA ratios (8.13 ± 0.00 and 1.12 ± 0.00, respectively), indicating a relatively higher proportion of unsaturated fatty acids compared to saturated fatty acids. This ratio is often used as an indicator of the overall healthiness of dietary fats. On the other hand, pitted TPOC has the highest ratio of n-6/n-3 fatty acids (11.30 ± 0.00), while COC 2 has the lowest (8.46 ± 0.00). A balanced ratio of n-6/n-3 fatty acids is important for maintaining optimal health, with excessive intake of n-6 fatty acids relative to n-3 fatty acids being associated with inflammation and various chronic diseases.

Table 4. Fatty acid profile of olive cakes (g/100 g).

Fatty Acid Profile	Olive Cakes					
	EOC 1	EOC 2	TPOC (Pitted)	TPOC (Dehydrated)	COC 1	COC 2
C13:0	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
C14:0	0.05 ± 0.00 ^{a}	0.05 ± 0.00 ^{a}	0.02 ± 0.00 ^{b}	0.03 ± 0.00 ^{b}	0.02 ± 0.00 ^{b}	0.03 ± 0.00 ^{b}
C16:0	10.63 ± 0.02 ^{d}	11.42 ± 0.00 ^{b}	11.28 ± 0.00 ^{c}	12.50 ± 0.00 ^{a}	10.68 ± 0.00 ^{d}	11.47 ± 0.02 ^{b}
C16:1n-7	0,49 ± 0.01 ^{c}	0.49 ± 0.00 ^{c}	0.45 ± 0.00 ^{d}	0.96 ± 0.01 ^{a}	0.53 ± 0.00 ^{b}	0.53 ± 0.01 ^{b}
C18:0	3.77 ± 0.03 ^{a}	3.33 ± 0.00 ^{c}	3.22 ± 0.00	2.44 ± 0.01 ^{e}	3.69 ± 0.00 ^{b}	3.25 ± 0.03 ^{d}
C18:1n-9	67.77 ± 0.02 ^{e}	66.08 ± 0.00 ^{f}	68.10 ± 0.00 ^{d}	68.50 ± 0.08 ^{c}	73.14 ± 0.00 ^{a}	71.46 ± 0.02 ^{b}
C18:1n-7	1.72 ± 0.04 ^{e}	1.95 ± 0.00 ^{d}	1.90 ± 0.00 ^{d}	3.06 ± 0.08 ^{a}	2.18 ± 0.00 ^{c}	2.42 ± 0.04 ^{b}
C18:2n-6	11.51 ± 0.00 ^{c}	12.61 ± 0.00 ^{a}	12.17 ± 0.00 ^{b}	9.88 ± 0.01 ^{d}	7.29 ± 0.00 ^{f}	8.39 ± 0.00 ^{e}
C20:0	0.61 ± 0.00 ^{a}	0.57 ± 0.00 ^{b}	0.47 ± 0.00 ^{c}	0.39 ± 0.00 ^{e}	0.48 ± 0.00 ^{c}	0.44 ± 0.00 ^{d}
C20:1n-9	0.39 ± 0.00 ^{a}	0.40 ± 0.00 ^{a}	0.34 ± 0.00 ^{b}	0.31 ± 0.01 ^{c}	0.28 ± 0.00 ^{d}	0.29 ± 0.00 ^{d}
C22:0	0.63 ± 0.00 ^{a}	0.43 ± 0.00 ^{b}	0.23 ± 0.00 ^{c}	0.00 ± 0.00 ^{e}	0.20 ± 0.00 ^{d}	0.00 ± 0.00 ^{e}
C22:1n-9	0.22 ± 0.00 ^{a}	0.12 ± 0.00 ^{c}	0.11 ± 0.00 ^{d}	0.03 ± 0.00 ^{e}	0.13 ± 0.00 ^{b}	0.03 ± 0.00 ^{e}
C24:0	0.21 ± 0.01 ^{b}	0.30 ± 0.00 ^{a}	0.15 ± 0.00 ^{c}	0.21 ± 0.01 ^{b}	0.13 ± 0.00 ^{c}	0.23 ± 0,01 ^{b}
ΣSFA	16.32 ± 0.06 ^{b}	16.56 ± 0.00 ^{a}	15.66 ± 0.00 ^{d}	15.83 ± 0.00 ^{c}	15.50 ± 0.00 ^{e}	15.70 ± 0.06 ^{d}
ΣMUFA	70.98 ± 0.05 ^{d}	69.30 ± 0.00 ^{e}	71.00 ± 0.00 ^{d}	72.90 ± 0.00 ^{c}	76.36 ± 0.00 ^{a}	74.72 ± 0.05 ^{b}
ΣPUFA	12.70 ± 0.01 ^{c}	14.14 ± 0.00 ^{a}	13.34 ± 0.00 ^{b}	11.28 ± 0.01 ^{d}	8.13 ± 0.00 ^{f}	9.58 ± 0.07 ^{e}
PUFA/SFA	0.78 ± 0.00 ^{d}	0.85 ± 0.00 ^{c}	0.85 ± 0.00 ^{c}	1.29 ± 0.00 ^{a}	0.53 ± 0.00 ^{e}	1.12 ± 0.00 ^{b}
n-6/n-3	10.57 ± 0.00 ^{b}	9.29 ± 0.00 ^{e}	11.30 ± 0.00 ^{a}	9.99 ± 0.00 ^{c}	9.73 ± 0.00 ^{d}	8.46 ± 0.00 ^{f}

EOC: Exhausted olive cake; TPOC: two-phase olive cake; COC: crude olive cake. SFA: Saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; the n-6/n-3 (∑ omega-6) (∑ omega-3). Mean ± standard deviation of determinations (n = 2). Different letters in the same column correspond to significant differences between species ($p < 0.05$). ANOVA followed by a post hoc Tukey test.

Approximately 85% of olive oil's composition comprises unsaturated fatty acids, with oleic acid being the predominant one, followed by saturated fatty acids [14]. The primary fatty acids present include a significant proportion of oleic acid (68.73%), which is a monounsaturated fat, along with other fatty acids like palmitic acid (12.28%), linoleic acid (2.80%), stearic acid (2.78%) and palmitoleic acid (0.97%). Various factors can influence changes in the fatty acid profile, thereby altering the composition of olive oil. External and intrinsic oxidative factors shape the oil profile, ultimately determining its sensory and functional attributes [68–70].

Furthermore, polyunsaturated fatty acids (PUFA), which are abundant in olive oil and consequently in pomace, are particularly susceptible to oxidation due to their chemical structure. This susceptibility makes them vulnerable to attack by free radicals, leading to the formation of volatile compounds that can alter the fatty acid profile of the oil [71].

The CF contents exhibit similarities among certain samples (TPOC: 9–10% and EOC: 1.5%), with the exception of COC, which displayed a variation content ranging from 8.43% to 14.5%. This divergence can be attributed to the different mills and extraction times from which these two cakes originated. This variability was also reflected in the fatty acid profile. As anticipated, COC 1 demonstrated an exceptionally high concentration of oleic acid, constituting 73.14% of the total fatty acids. However, considering oxidative stability, it is noteworthy that the total polyunsaturated fatty acid (PUFA) content is 9%. This observation aligns with findings reported by other researchers [45,72]. Analysing the fatty acid profile of the olive cake by-product, a prominent abundance of monounsaturated fatty acids is evident, with oleic acid (C18:1n-9) being predominant, accounting for approximately 69% of the total. Additionally, linoleic acid (C18:2n-6), palmitic acid (C16:0) and stearic acid (C18:0) were among the other major compounds identified. Trace amounts of various other fatty acids were also detected. Collectively, these compounds represent approximately 98% of the total fatty acids, which is consistent with previous findings in the literature concerning olive products and by-products. [73,74].

This discrepancy can be attributed to the defatting process applied to the olive by-product used in this study, a procedure commonly conducted in some mills to reduce the total fat content, which can also impact the FA profile. However, compared to findings from some authors who studied partially defatted extracted olive pomace, our values were lower [67]. The ginning process may account for this variance, as a significant portion of the fruit's oil is retained in the stone during this process.

In terms of the fatty acid (FA) content of EOC, once again, oleic acid emerges as the primary fatty acid. EOC exhibited the lowest FA content compared to the other olive cakes, particularly in terms of oleic acid and monounsaturated fatty acids (MUFA). This discrepancy can be explained by the fact that the olive by-product used in this study was defatted, a procedure carried out in some mills that reduces total fat and can also affect the FA profile. However, compared to findings from some authors who studied partially defatted extracted olive pomace, our values were lower [67]. The ginning process may account for this variance, as a significant portion of the fruit's oil is retained in the stone during this process [75].

4. Conclusions

The comprehensive examination of olive pomace's chemical composition, as presented in this study, unveils a nuanced and captivating array of its attributes. The interplay between processing methods and olive maturity levels profoundly influences moisture content, crude fat, neutral detergent fibre, fatty acids, phosphorus and phytic acid levels, showcasing the diverse properties inherent in this by-product. Rather than posing a challenge, this variability opens avenues for olive cake utilisation across multiple sectors. Compared to other foods like cereals and pulses, olive cake boasts a relatively low phytic acid content. By discerning the phytic acid content in different types of olive cake and understanding their chemical and physical traits, we can tailor processing methods to specific applications. This approach minimises antinutritional effects while maximising

the by-product's value. Further studies delving into optimised processing techniques, strategies for reducing phytic acid content and the exploration of innovative applications for olive cake are crucial. Such endeavours are pivotal in valorising this by-product and fostering sustainability within the olive oil industry. Expanding research efforts in these areas will not only enhance our understanding of olive cake but also contribute significantly to advancing sustainability practices in the olive oil sector.

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