Jordan Journal of Pharmaceutical Sciences, Volume 18, No. 4, 2025

Elemental Analysis of Olives' Fruits Using Synchrotron Radiation X-Ray Fluorescence (XRF)

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ABSTRACT

Olive-trees (*Olea europaea* L.) are the dominant trees cultivated in the Mediterranean basin. X-ray fluorescence (XRF) is one of the sensitive, rapid and simple analytical techniques to study the essential element content of medicinal plants. This project aimed to apply XRF in the determination of metallic elements content of olive fruits. Herein, the trace elements in *O. europaea* L. fruits have been analysed using XRF spectrometry. Samples were collected from four environmentally different areas and subjected to X-Ray fluorescence detection and mapping using a photon flux of 10^8-10^9 photons in the energy range between 3.65-14 keV. The results showed that the olive fruits are rich in nutritional elements like K, Ca, Mn, Fe, Cu and Zn. It can be concluded that XRF could be considered a valuable technique for detection and mapping the elemental contents of olive fruits.

Keywords: *Olea europaea* L, Trace elements, Synchrotron, X-ray fluorescence, Elemental Analysis.

INTRODUCTION

Throughout the world, plants provide food and medicine to people and animals. Additionally, plants are considered a rich resource for the pharmaceutical and cosmetic industry [1]. The olive tree (*Olea europaea* L.) is one of the most ancient domestic and cultivated plants of the Mediterranean basin and has an important economic impact in the production of olive oil [2]. Kaniewski et al. (2012) discussed thoroughly the origin, domestication and importance of the olive tree in different cultures of the Middle East starting from the prehistorical times [3]. Since time immemorial, people in the Middle East specifically, have used olive trees (fruits, oil, and the prepared extracts of the leaves) for their health benefits and in fighting numerous diseases from simple infections and common colds to lowering cholesterol and preventing heart diseases. Olives and olive oil are considered as the primary constituent of the so-called healthy "Mediterranean diet" [4-6]. Olives are a rich source of important constituents, such as sterols, tocopherols, carotenoids and minerals in addition to their principal components including water (60–75%), lipids (10-25%), reducing sugars (2–5%) and phenolic substances (1–3%) identified mainly as flavonoids and anthocyanins [7-10]. Several studies describe the anticancer and cardioprotective effects of fruits, oil, and the leaves of olives (11-13). Also, the antioxidative potential and the protective effect of the phenolic compounds of *O. europea* against radiation and photocarcinogenesis are reported [14]. Alzweiri & Al-

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Received: 03/08/2024 Accepted: 03/11/2024.
DOI: https://doi.org/10.35516/jjps.v18i4.3099

Hiari (2013) reported the successful quantification of hydroxytyrosol in olive leaf extracts using a validated HPLC method, underscoring the importance of Jordanian olives in antioxidant research [15].

In the recent years, the determination of macro- and micro-elements, including metallic components, gained high importance. The absence or accumulation of these metallic components determines the quality of plant products which in turn affects the health of the humans. Moreover, identification and quantitation of trace elements using different methods became a legal procedure in determining the geographical origin and the quality of plant-based products [16-18]. The accumulation of micronutrients (like Fe, Mn, and Zn in crop plants) has potentially important consequences in crop production as well as in the etiology and prevention of chronic diseases in humans. For example, the lack of Zn in human food leads to a wide range of health troubles including weaknesses in the immune system, learning ability and physical growth in addition to an increase in mortality [19, 20].

Several researchers from different countries examined metals and trace elements in olive oil, olive fruits and soil samples [21-24]. To the best of our knowledge, no study has been carried out with olive fruits grown in Jordan in this aspect, although researchers from Jordan emphasize the chemical and elemental profiling of the native plants from the flora of Jordan [25]. In a very recent study Alakayleh (2025) reported the adsorption behavior of diclofenac onto olive leaf-derived adsorbent [26]. Hence, the objective of the current study was to apply XRF in determination of the presence of metallic elements (K, S, Ca, Mn, Fe, Ni, Sr, Cl, Kr, Ar, Cu, Sc) in olive fruits collected from four different locations of the Mediterranean biogeographic zone of Jordan to highlight the nutritional value of olive fruits and to compare the effect of the environmental factors on their occurrence. Some of these trace elements are essential for human nutrition; Ca, Cr, Cu, Fe, K, Mn, Ni, Zn, while others, such as Cu, Fe, Ni, Zn and Mn play an important role in oxidative stability. Additionally, this study aimed to evaluate the importance of XRF technique in elemental analysis of the medicinal plants.

MATERIALS AND METHODS

Samples

Four different samples of olives grown in the Mediterranean biogeographic zone in Jordan were collected. Fully grown fruits were hand-picked from trees naturally distributed in selected regions of the country which were subsequently exposed to different environmental factors: Sample 1: from a farm in a remote area; Sample 2: from a farm next to the industrial area; Sample 3: from the countryside, less affected from traffic (no adjacent industrial complex); Sample 4: from Amman living area garden adjacent to a street with heavy traffic. It is worth mentioning that the olives from the selected sample trees get collected by the inhabitants of each area either for oil production or for consumption as a condiment. The collected plant materials were identified by Prof. Barakat Abu Irmailah (School of Agriculture, The University of Jordan). Voucher samples have been kept in the Department of Pharmaceutical Sciences, School of Pharmacy, The University of Jordan (FMJ-OLE-XS1, FMJ-OLE-XS2, FMJ-OLE-XS3, FMJ-OLE-XS4).

The fruits were carefully washed with distilled water to remove the surface dust. Then, thick transversal cuts were done in the middle of the fruits. An area of 20x20 mm was subjected to XRF analysis.

Instruments

Samples were subjected to X-Ray Fluorescence detection and mapping. XRF data were collected at BESSYII, Germany. The ring was operating in top-up mode with a current of ~309 mA. The beamline provided a photon flux of 10^8-10^9 photon/s in the energy range between 3.65-14 keV. A Si (111) double crystal monochromator of 1.4×10^{-4} resolving power was used to monochromatize the incoming radiation from the bending magnet source and ensure the spectral purity, respectively.

A beam size of 5x5 µm2 was obtained using a Polly capillary X-ray optics to ensure a higher flux density on the sample [27]. The experimental setup was equipped with a multi-axis motorized sample manipulator which provided different degrees of freedom to align the sample surface relative to the incident beam. The sample was positioned at 45 degrees from the incident beam.

A silicon drift detector (SDD, Bruker Nano GmbH, XFlash 5030, 131 eV energy resolution at Mn-Kα) with a 30 mm^2 active area was used to collect the emitted radiation. The distance between the sample and detector was set to 15 mm. For the acquisition of the elemental maps, the excitation energy was fixed to 12.5 or 13.6 keV. A step size of 250 µm (horizontally) x 80 µm (vertically) was applied for mapping the samples, with a 10 sec integration time.

RESULTS AND DISCUSSION

Currently, several methods and techniques are available for the qualitative and (semi)quantitative determination of trace elements in plant materials namely, inductively coupled plasma atomic emission spectrometry (ICP-AES), atomic absorption spectrometry (AAS), neutron activation analysis (NAA), energy dispersive X-ray fluorescence (ED-XRF) and X-ray fluorescence (XRF) [22, 28-31]. X-ray fluorescence has several advantages over other analytical techniques used for the detection and analysis of trace elements in medicinal plants and is well suited for multi-elemental determinations in plant samples. Moreover, XRF is one of the sensitive, rapid and simple analytical techniques to study the essential element content of medicinal plants [32]. The samples do not need any chemical treatment, and any possible contamination is therefore avoided [33]. XRF requires only a small amount of test material therefore minimizing the need for extensive sample preparation. Additionally, it is a non-destructive technique meaning that the sample remains intact after analysis. This is particularly beneficial when dealing with rare or precious medicinal plants. Unlike other techniques that often require complex and time-consuming sample preparation, XRF typically requires only basic samples [18, 34]. These advantages make XRF a powerful tool for the analysis of trace elements in medicinal plants.

In the present study, all collected samples were not artificially irrigated since they were belonging to the natural flora of the locations from which they were collected. While all locations belong to the same biogeographic zone; they differed slightly in the altitude of the collection sites and their vicinity to different polluted areas.

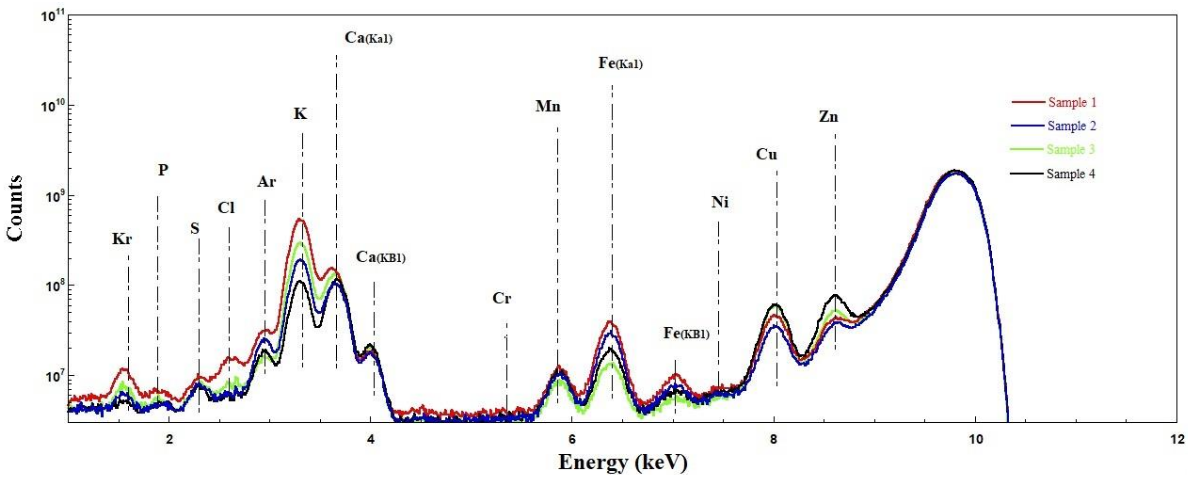

Figure 1 Relative abundance of metals in the four analyzed samples

Based on the findings of the XRF spectrometry and as shown in Figure (1), the olive fruit is rich in many nutritional elements regardless of the environmental factors influencing their growth. The relative amounts of the major elements in the four analysed samples collected from four environmentally different locations revealed approximately equivalent inorganic profiles. In all samples, K was the major element followed by Ca, Zn, Fe, Cu, Zn and Mn. However, sample 1(collected from the farm in a remote area) exhibited the highest concentration in all elements except Cu and Zn.

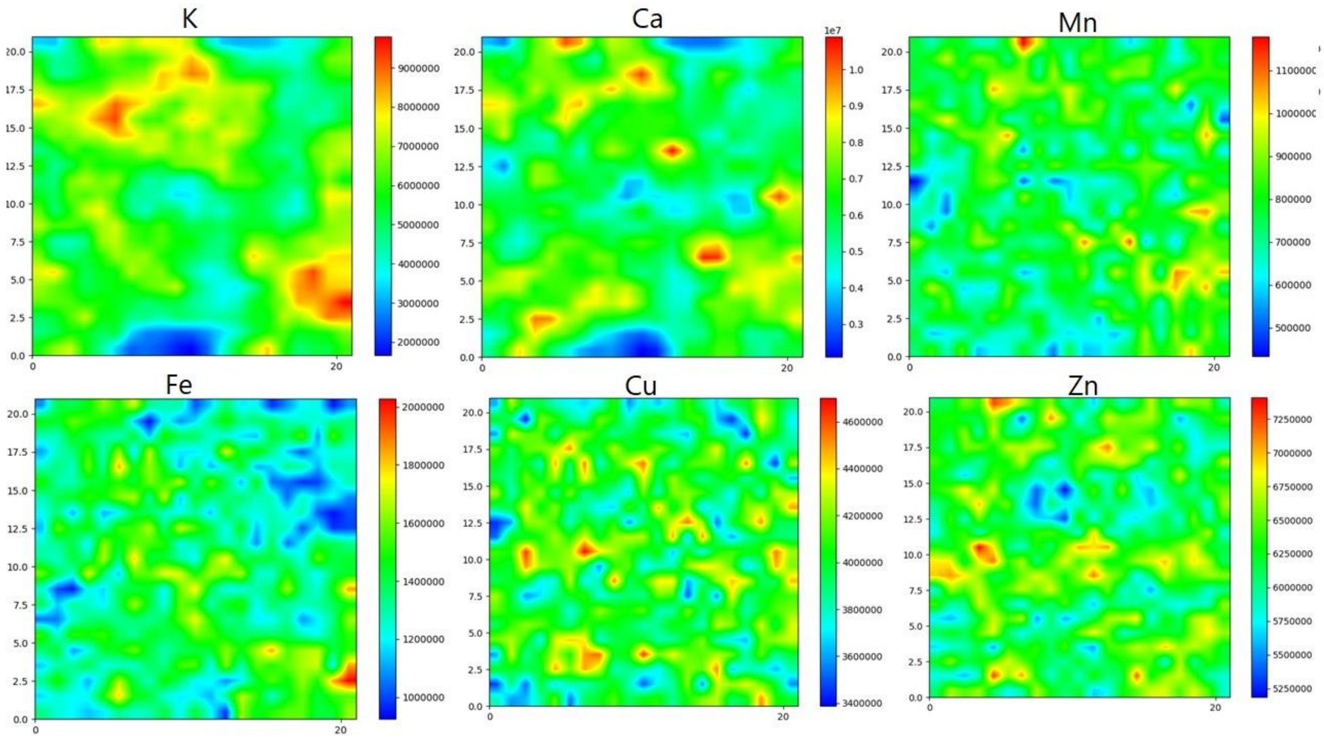

Figure 2 Heat images of the olive sample 1 for K, Ca, Mn, Fe, Cu and Zn

Another application of the XRF spectrometry is the presentation of the heat images of the different elements. Figure 2 shows the exact positions of K, Ca, Mn, Fe, Cu and Zn, as well as their relative abundances throughout the surface of the olive sample. In Figure 2, the image of the sample 1 is presented as an example. The same experiment was repeated with the other three samples and similar findings were detected.

In a study conducted in Algeria to analyze the trace metal contents in the leaves and fruits of the olive trees, it was observed that the concentrations of Fe and Mn were higher in the leaves compared to the fruits [25]. These findings highlighted that the nutrient value of this plant is not only in the fruits but also in the leaves of the olive tree. The elevated levels of these metals in the leaves confirm their significant role in the plant's physiology as well as in medicine. The authors revealed that in the olive fruits, K was the main metal present followed by P and Fe [25]. In agreement with the reported findings of Nedjimi (2020), K was detected in all studied fruit samples as the main element in the current study as well.

On the other hand, several researchers identified the occurrence of trace metals in olive oil using similar analytical methods [35, 36]. Applying ED-XRF as the method of analysis, Frederic et al. (2020) detected Fe, Cr, Zn, Cu, Ni and Co as trace elements in olive oil. Luka and Akun (2019) identified Cu as the highest trace element in olive oils collected from different markets in Northern Cyprus using ICP-MS [36].

These variations in metal content across different studies suggest that environmental factors such as soil composition, climate and agricultural practices can significantly influence the trace metal content in plants. The differences in metal concentration between the leaves, fruits and oil also underline the importance of considering both plant parts and oil of *O. europea* in nutritional and medicinal evaluations.

CONCLUSIONS

The levels of K, Ca, Mn, Fe, Cu and Zn in *O. europaea* indicate that the plant is a notable source of nutrient elements with potential therapeutic benefits. This further suggests that the plant material may be useful in the formulation of nutritional and/or therapeutic products. The present study reported, for the first time, the determination of the above mentioned nutrient elements in the olive fruits which collected from the trees grown in different locations of the Mediterranean biogeographic zone in Jordan. The applied analytical method XRF is one of the most sensitive, rapid and simple analytical techniques for the elemental analysis of medicinal plants. XRF technique can be considered as a suitable methodology for mineral determinations in olive samples since the analyzed samples do not need any chemical treatment ensuring the preservation of all the sample's chemical properties.

Conflicts of Interest:

The authors declare no conflict of interest.

Funding:

The authors received no funding for this research.

Data availability:

The data are available upon request.

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التحليل العنصري لثمار الزيتون باستخدام تقنية الأشعة السينية الفلورية بالأشعة السنكروترونية (XRF)

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ملخص

تُعد أشجار الزيتون (*Olea europaea* L.) من أكثر الأشجار زراعةً في منطقة حوض البحر الأبيض المتوسط. تُعد تقنية الأشعة السينية الفلورية (XRF)واحدة من التقنيات التحليلية الحساسة والسريعة والبسيطة لدراسة محتوى العناصر الأساسية في النباتات الطبية. هدف هذا المشروع إلى تطبيق تقنية XRFفي تحديد محتوى العناصر المعدنية في ثمار الزيتون. تم تحليل العناصر النزرة في ثمار *O. europaea* L. باستخدام مطياف الأشعة السينية الفلورية. جُمعت العينات من أربع مناطق بيئية مختلفة، وخضعت للكشف ورسم الخرائط بالأشعة السينية باستخدام تدفق ضوئي يتراوح بين 10^8–10^9 فوتونات، ضمن نطاق طاقة بين 3.65 و14 كيلو إلكترون فولت (keV). أظهرت النتائج أن ثمار الزيتون غنية بالعناصر الغذائية مثل البوتاسيوم (K)، والكالسيوم (Ca)، والمنغنيز (Mn)، والحديد (Fe)، والنحاس (Cu)، والزنك (Zn).يمكن الاستنتاج بأن تقنية XRF تُعد وسيلة قيّمة للكشف ورسم خرائط المحتوى العنصري في ثمار الزيتون.

الكلمات الدالة: *Olea europaea* L، العناصر النزرة، السنكروترون، الأشعة السينية الفلورية، التحليل العنصري.

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تاريخ استلام البحث 2024/08/03 وتاريخ قبوله للنشر 2024/11/03.