



Original article

First evidence of fractionation on stable organic carbon isotopes in ancient Egyptian linen textile fibers during degradation

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ARTICLE INFO

Article history:

Received 26 April 2023

Accepted 4 August 2023

Keywords:

Ancient Egyptian linen

Stable carbon isotopes

Fractionation

Aging

Degradation

ABSTRACT

The study of the fractionation of the stable carbon isotopes ^{13}C and ^{12}C through the $\delta^{13}\text{C}$ value finds applications in various fields of science, from quality control in agribusiness to the study of the geographical origin of materials. At the plant level, this information is useful to prove which photosynthetic cycle a particular plant belongs to: C3, C4 or Crassulacean Acid Metabolism. In this work, the Isotopic Ratio Mass Spectrometry technique was used to shed light and investigate about the degradation due to aging of an ancient linen textile of Egyptian origins, in order to see whether degradation phenomena change the content of stable carbon isotopes, over time. For this purpose, the $\delta^{13}\text{C}$ values of the ancient sample were compared with contemporary Egyptian and French flax fibers samples. For the first time, it was observed that, contrary to what might have been expected, material degradation due to natural aging contributes to the phenomenon of the isotopic fractionation, due to the increasing in measured $\delta^{13}\text{C}$ values from contemporary to ancient samples.

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Introduction

The relative abundance of the natural carbon isotope ^{13}C and ^{12}C in plant tissues is a simple and reliable indicator of the pathway by which carbon has been fixed by a plant. Depending on the enzymes involved in photosynthesis, plant species can be distinguished by their $\delta^{13}\text{C}$ value [1]. In general, plants belonging to the C3 cycle, also known as the Calvin cycle, have a $\delta^{13}\text{C}$ values between -22‰ and -32‰ , while plants in the C4 cycle, or Hatch-Slack cycle, have $\delta^{13}\text{C}$ values between -8‰ and -18‰ [2]. There is another category of plants called CAM, referring to the Crassulacean Acid Metabolism, where $\delta^{13}\text{C}$ ranges from -10‰ to -20‰ [3]. However, there are environmental variables that cause slight variations in $\delta^{13}\text{C}$ values within the same family [4]. Firstly, a source of isotopic discrimination is provided by the effect of soil fertilization; it was noted that plants grown in highly fertilised

soils showed more positive $\delta^{13}\text{C}$ values than those grown in conditions of nitrogen and potassium deficiency, leading to differences of $\pm 2\text{‰}$ [5]. Secondly, temperature variations due to different plant growth conditions were evaluated, but there is no total consistency with the scientific literature; if the effect of temperature is indeed real, then it can be considered negligible [6]. Different parts of the plant also have different isotopic contents, therefore analyses in the leaves rather than in the roots will lead to results that may differ by as much as 2‰ [7,8]. The most interesting factor, however, is the difference in carbon isotope content between the different macromolecules that make up the same plant [9]. Cellulose, hemicellulose, and lignin are the main components of a plant fiber. The $\delta^{13}\text{C}$ values for hemicellulose compared with cellulose differ by more than $\pm 1.5\text{‰}$, while lignin is depleted of the heavier isotope, compared with cellulose, by $2\text{--}4\text{‰}$, representing the main source of differences in isotopic content [10]. Deterioration of plant fibers is related to cellulose degradation because is the main component of their structure [11–15], giving rise to low molecular weight and volatile species that produce a depletion of carbon, oxygen and hydrogen atoms. Therefore, deterioration represents one of the most

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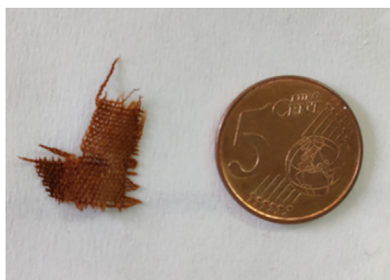


Fig. 1. A piece of the Ancient Egyptian linen textile fabric collected during the Cairo University Excavation Season in Saqqara, in the tomb of Ptahmes, mayor of Memphis and high-ranking official under the Pharaohs Seti I and Ramses II of the XIX Dynasty.

interesting to study from the point of view of isotopic abundance. Indeed, different decomposition phenomena in plant fibers appear to induce fractionations in the isotopic composition of the carbon dioxide developed during artificial degradation [16]. However, when we are performing stable carbon isotopic measurements on historical samples, there is a big challenge to overcome: the variation of the carbon isotopic abundance on the atmospheric CO_2 over time due to the human activity. Since the Industrial Revolution the massive burning of fossil fuels has led to significant changes in the carbon isotopic content of atmospheric CO_2 . Fossil fuels are depleted in ^{13}C , with a $\delta^{13}\text{C}$ that ranges from -44% to -19% [17], more negative than the $\delta^{13}\text{C}$ of the atmospheric CO_2 (about -8.4% to the present) [18]. Therefore, the combustion of fossil fuels increases the $^{12}\text{CO}_2$ at a faster relative rate than $^{13}\text{CO}_2$ [19]. This dilution effect, known in literature as the ^{13}C Suess Effect [19–21], aims to rationalize the gap in the $\delta^{13}\text{C}$ value of CO_2 , from pre-1760 BP to the present day. It was observed that CO_2 become more depleted in ^{13}C from the Industrial Revolution to our days, reflecting the strong changes in the $\delta^{13}\text{C}$ value, from -6.4% (pre-1760 atmosphere) to -8.4% (contemporary atmosphere), due to increased anthropogenic fossil fuel emissions [19]. Since the carbon isotopic composition of the atmospheric CO_2 greatly impacts the biological carbon cycle, it was proposed by J. Dombrosky, a Suess effect correction, in order to compare samples from different eras [18].

Anyway, until now no measurements on linen fibers about the differences in the $\delta^{13}\text{C}$ values from historical to contemporary samples, have been done. Because in the past, linen, a C_3 plant [22], was the most important source of materials to make fabrics, several questions require answer. Are there any differences in carbon isotopic composition upon changing geographic region of cultivation? Any differences between the extracted pure cellulose and the total fiber carbon content? Can degradation processes due to aging influence the $\delta^{13}\text{C}$ value? So, taking note of the phenomenon of isotopic fractionation, the present work aims to investigate the degradation process of ancient flax textile fibres of Egyptian origins by studying the $\delta^{13}\text{C}$ values over time, by comparison with modern flax fibers of Egyptian origins. To make independent the isotopic measurements from the different latitude geographic areas, with different soil, and generally different climate, contemporary flax fibers from French Provence were studied and compared with the Egyptians samples. Moreover, the Brendel method was used to purify the content of cellulose in the fibers samples from hemicellulose and lignin [23]. Finally, the $\delta^{13}\text{C}$ values coming from the historical samples, were corrected from the Suess effect. In addition, the results were compared with the raw materials in order to evaluate the role of the extraction step on the isotopic ratio. The ancient textile (Fig. 1) was collected during the Cairo University Excavation Season in Saqqara in the tomb of Ptahmes, mayor of Memphis and high-ranking official under the Pharaohs Seti I and Ramses II of the XIX Dynasty. The sample analyzed in this study

was discovered during the 2013/2014 Cairo University excavation. While the team was removing the sand lying against the southern outer wall of the tomb, they found many broken jars. Inside a jar the archaeologists found the remnants of a canvas fabric; such material may have been used to cover the original contents of the jar, which turned out to be one of the oldest cheeses in the world, studied in our previous work [24].

Research aim

The aim of this work is to shed light on the degradation contribute due to natural aging of ancient linen textiles fibers on the $\delta^{13}\text{C}$, in order to invite researchers to take into account this aspect when historical linen samples are studied from an isotopic perspective.

Materials and methods

In this work the measurements carried out on different types of samples are reported. A first set of samples consisted of contemporary flax fibers from two different climatically regions: French Provence and Lower Egypt. A second set, on the other hand, consisted of the ancient linen found in the tomb of Ptahmes and dating back to the 19th Dynasty. To perform the isotopic investigation by Isotopic Ratio Mass Spectrometry (IRMS) both sets were analyzed after appropriate washing procedures and, subsequently, cellulose extraction processes were performed on them in order to verify any differences.

Chemicals

Hydrochloric Acid 37% (HCl), Acetic Acid 99.9% ($\text{C}_2\text{H}_4\text{O}_2$), Nitric Acid 69% (HNO_3), Absolute Ethanol ($\text{C}_2\text{H}_6\text{O}$) and Sodium Dodecyl Sulfate (SDS), were purchased from Sigma Aldrich-Merck, Italy. All chemicals are analytical grade. Dilutions and solubilisations were carried out with MilliQ H_2O .

Washing stage

This step was included in the experimental procedure because the ancient sample was contaminated by materials containing both inorganic carbon (Natron, $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$) and organic carbon, i.e. traces of protein from the cheese contained in the wrapping fabric [24]. Considering the nature of these contaminants, it was considered suitable to design a targeted system for washing the fabrics consisting of four steps: the first step (x3) involves treatment with MilliQ H_2O in an ultrasonic bath at a temperature of $40\text{ }^\circ\text{C}$ for 3 min. During this step, all water-soluble impurities such as Natron was removed. This is followed by the second step (x3), in which the tissue is treated with a 0.1 M HCl solution in an ultrasonic bath at $40\text{ }^\circ\text{C}$ for 3 min. The use of hydrochloric acid is required to denature any remaining protein traces between the fibres and pass them into solution; it also promotes the solubilisation of any less soluble inorganic residues. The third step involves washing with a surfactant (SDS 1%) in an ultrasonic bath at $40\text{ }^\circ\text{C}$ for 3 min. This is used to remove any traces of non-polar residues. Finally, the fourth step (x6), acts as a rinse to remove residual SDS and involves a treatment in H_2O at $40\text{ }^\circ\text{C}$ for 3 min. The cleaned fibers were dried using a nitrogen flow. The effectiveness of the washing stage was evaluated by ATR-FTIR spectroscopy comparing the fibers before and after the treatment (Fig. 2).

Cellulose extraction

For the extraction step of the two different matrices, the Brendel method was adopted, a well-known protocol in the literature

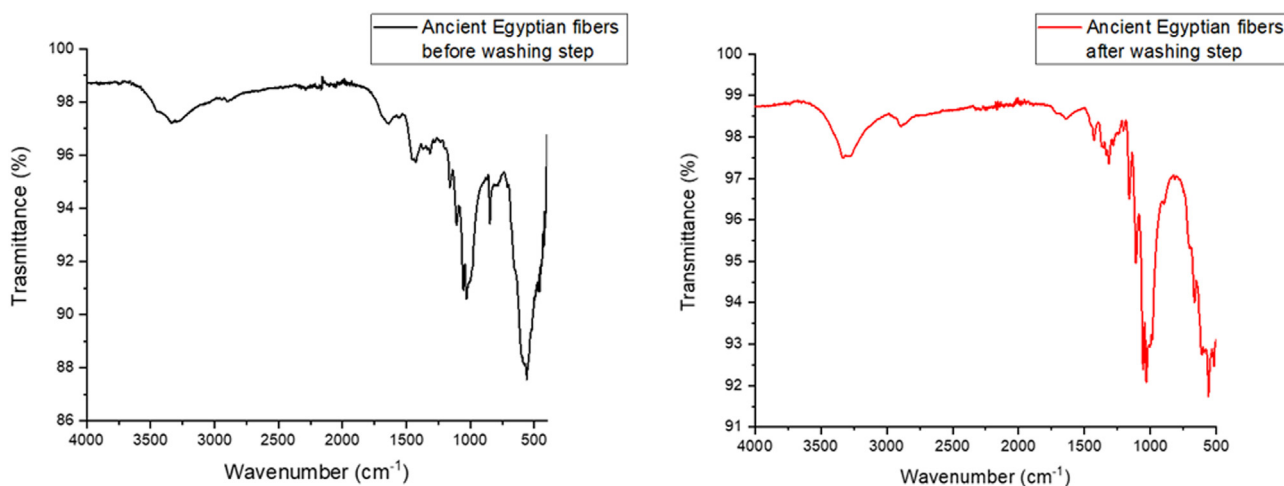


Fig. 2. ATR-FTIR spectra of the Ancient Egyptian linen fabric before and after the washing step. In the cleaned fibers, the absence of the band related to the Na_2CO_3 around 700 cm^{-1} is observed.

for isolating cellulose from non-cellulosic macromolecules such as lignin and hemicellulose. This method was designed to target pure cellulose extracts for isotopic abundance analysis. The three matrices underwent the same extraction treatment. 10 mg of the sample, defibrated using metal tweezers, were transferred to a 10 ml glass tube. Subsequently, 2.0 ml of acetic acid (80% v/v), 0.2 ml of 69% HNO_3 were added and, after capping the tube with a glass stopper, it was transferred into an oven heating chamber at $120\text{ }^\circ\text{C}$ for 20 min. After cooling, 2.5 ml of absolute ethanol were added and centrifuged for 5 min at 2000 rpm. After removal of the supernatant, the bottom body was washed as follows: 2×2.5 ml absolute ethanol to remove extraction waste products; 2×2.5 ml H_2O to remove traces of nitric acid; 2×2.5 ml ethanol. The last step in Brendel's method involves 2×2.5 ml with acetone to promote water removal. In this procedure, to avoid carbon contamination by acetone, the last step has been replaced with 2×2.5 ml H_2O . The washes, after the addition of the aliquot of the various solvents, included a centrifugation step for 5 min, 2000 rpm. The cellulose sample obtained was placed in a vacuum drying oven at a temperature of $35\text{ }^\circ\text{C}$ overnight, to remove all traces of water and residual solvents.

Textiles and extracts characterization

The chemical properties of the textiles and cellulose extracts were assessed using standard surface characterization techniques. The infrared spectra were obtained by Fourier transform techniques with a PerkinElmer Spectrum Two FT-IR spectrometer with ATR equipment. Each spectrum was acquired across the wavenumber range of $450\text{--}4000\text{ cm}^{-1}$. Resolution ratio was 4 cm^{-1} . Moreover, to identify microscopic characteristics of the raw materials, a Zeiss Supra 55VP with a field emission gun was used to perform SEM measurements.

Isotopic ratio mass spectrometry (IRMS)

The $\delta^{13}\text{C}$ values were measured directly in the fibers' samples and in the cellulose from raw fibers using an isotope ratio mass spectrometer Delta V Thermo Advantage Isotope Ratio MS, Bremen, Germany, equipped with an elemental analyzer Flash EA Thermo 1112 Series and a combustion reactor filled with metallic copper/copper oxide. Three independent weighed quantities were performed for each sample (200–1000 μg) and placed in tin capsules, to measure both the $\delta^{13}\text{C}_{\text{fiber}}$ and $\delta^{13}\text{C}_{\text{cellulose}}$. The isotopic

ratio was expressed in $\delta\text{ }^\circ\text{‰}$ vs V-PDB (Vienna-Pee Dee Belemnite) for $\delta^{13}\text{C}$, according to the following formula:

$$\delta(\text{ }^\circ\text{‰}) = \left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 1000$$

where R_{sample} is the isotope ratio measured for the sample and R_{standard} is the isotope ratio of the international standard. The isotopic values were calculated vs casein "CAS line 10", a working in-house standard which was itself calibrated against USGS 40 L-Glutamic Acid international reference material.

Isotopic ratio mass spectrometry measurements were conducted for each sample in order to investigate three main aspects: Firstly, the mean $\delta^{13}\text{C}$ values obtained from the raw fibers were compared with the corresponding values of the cellulose extracts, in order to check whether the content of macromolecules such as lignin and hemicellulose causes changes in $\delta^{13}\text{C}$ values; secondly, $\delta^{13}\text{C}$ mean values from raw fibers and cellulose extracts of Egyptian and French contemporary samples were compared in order to test whether the different latitude affects the $\delta^{13}\text{C}$ value. Finally, by comparing the $\delta^{13}\text{C}$ values of ancient and modern Egyptian samples, it was verified if the degradation process due to the aging of the linen fibers, contributes to the isotopic fractionation of the organic carbon.

Statistics

All data were analysed with Microsoft Excel 2021 (Microsoft Office 365) and OriginLab 2018 (OriginLab, Northampton, MA, USA). Three values of $\delta^{13}\text{C}$ (‰) were obtained from each sample, from which the mean and the associated variance were calculated. To assess the differences in the $\delta^{13}\text{C}$ values of each sample, the means were compared using the Student's t-Test assuming equal variance after verifying the condition via Fisher Test. Statistical p-values were set significantly at 95% confidence level for both Student's t-Test and Fisher Test.

Result and discussion

Textiles and extracts characterization

Scanning electron microscopy

The morphology of fibers and fabric surfaces was investigated by Scanning Electron Microscopy. The analysis of the images allows to identify the materials as pure flax, due to typical nodes of

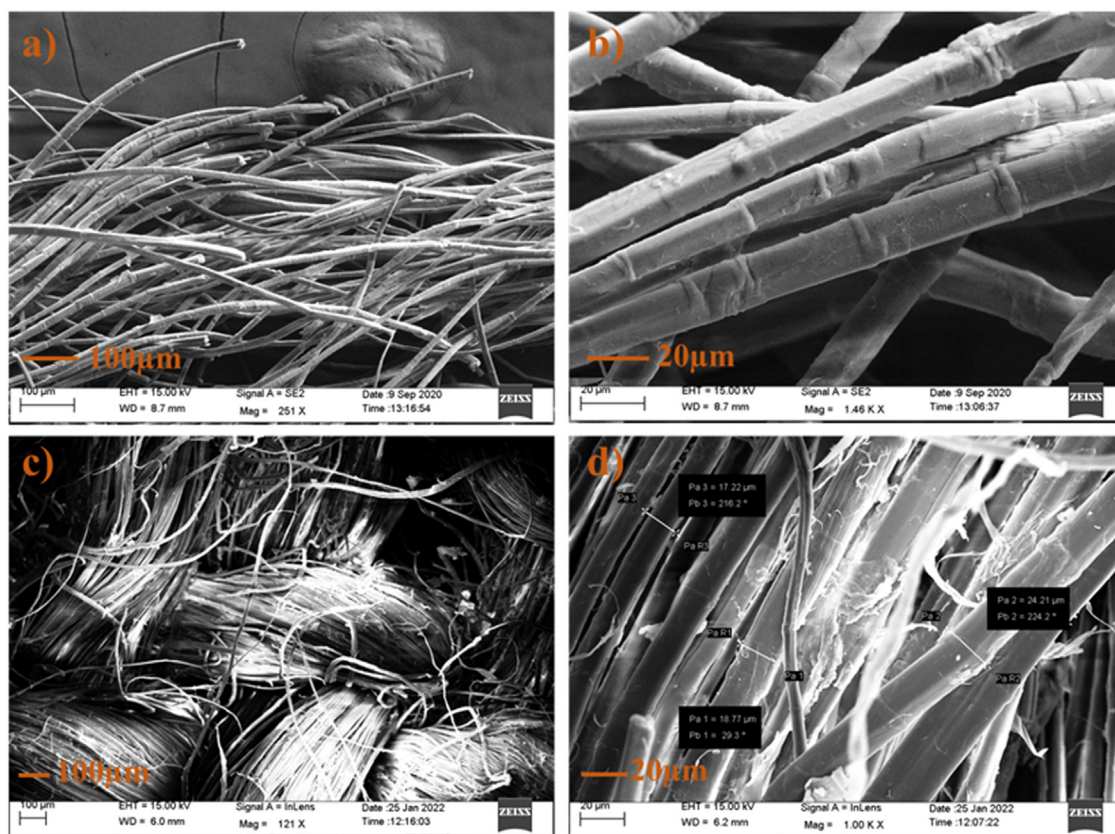


Fig. 3. SEM images of the Ancient Egyptian linen fibers (a, b) and Contemporary Egyptian linen fibers (c, d). In both case the fibers diameter is between 14 μm and 24 μm .

linen [25–28], which can be clearly seen in Fig. 3. The structure of the fabric is made up of many cylindrical fibers ranging from 17 μm to 24 μm , with the characteristic "bamboo cane" shape that distinguishes linen fibers.

ATR-FTIR analysis

A non-invasive method that was mostly used to identify the textiles is ATR-FTIR spectroscopy, useful to identify the material from a chemical point of view [28] and to evaluate the success of the cellulose extraction step. The spectra are reported in Fig 4. All the spectra show the same bands typical of cellulose, as flax is a plant consisting mainly of cellulose. At 3310 cm^{-1} the stretching of hydroxyl groups typical of cellulose can be observed, while at 2800 cm^{-1} the stretching of $-\text{CH}_2-$ bonds is present. Only in the old sample, at 1710 cm^{-1} , it is possible to see a small broad peak, attributable to the $\text{C}=\text{O}$ bond of a carbonyl group, due to the degradation of the fibers. At 1637 cm^{-1} there is a weak signal due to the presence of water [29]. The peaks at 1426 and 825 cm^{-1} are due to the crystalline and amorphous domains of the cellulose, respectively [30]. At 1364 and 1278 cm^{-1} , bending modes ($\text{H}-\text{C}-\text{C}$) of the cellulosic structure can be observed [31,32], while at 1335 and 1315 bendings of the $\text{C}-\text{OH}$ bonds are present. In addition, there are several peaks between 1200 and 900 cm^{-1} , corresponding to $\text{C}-\text{O}-\text{C}$ and $\text{C}-\text{OH}$ stretching. In particular, the $\text{C}-\text{O}-\text{C}$ stretching vibration of the β -glycosidic bond is observed at 1160 cm^{-1} ; the peaks at 1109 cm^{-1} and 1053 cm^{-1} are assigned to $\text{C}-\text{OH}$ secondary alcohols groups, and at 1030 cm^{-1} there are the vibrations of primary alcohols [28,30–33]. No lignin peaks around 1452–1454 cm^{-1} were seen. From the spectra of the cellulose extracts of the two samples, there is no substantial difference; this confirms the fact that the flax textile fibres consist mainly of cellulose [18]. The only difference is the presence of a peak at 1732 cm^{-1} , due either

Table 1

$\delta^{13}\text{C}$ mean values of the analyzed raw fibers and cellulose extracts samples.

Samples	$\delta^{13}\text{C}$ values (‰)	
	Raw fibers	Cellulose extract
Ancient Egyptian linen	-24.92 ± 0.28	-24.96 ± 0.10
Contemporary Egyptian linen	-28.25 ± 0.10	-28.14 ± 0.10
Contemporary French linen	-27.91 ± 0.18	-28.47 ± 0.13

to a residual acetic acid used during the extraction phase, or to an acetylation reaction in an acid environment. This suggests that the presence of lignin could be neglected because its concentration in these fibres is certainly very low and, in any case, below the limit of detection of the ATR-FTIR technique.

Isotopic ratio mass spectrometry (IRMS)

The $\delta^{13}\text{C}$ mean values obtained for the six matrices analysed are summarized in Table 1. The results will be discussed in three sections: firstly, the Student's t-Test was carried out by comparing the means of the $\delta^{13}\text{C}$ values relating to the crude fibers with the means relating to the cellulose extracts. For the ancient Egyptian samples, the $\delta^{13}\text{C}$ mean values for the raw fibers and the cellulose extract are -24.92% ($\sigma = 0.28$; $n = 3$) and -24.96% ($\sigma = 0.10$; $n = 3$), respectively. About the contemporary Egyptian samples, the $\delta^{13}\text{C}$ mean values for the raw fibers and cellulose extract are -28.25% ($\sigma = 0.10$; $n = 3$) and -28.14% ($\sigma = 0.10$; $n = 3$), respectively. For both contemporary and ancient samples, the p value from the Student's t-Test being greater than 0.05; it can be stated that there is no appreciable difference between fibers and cellulose extracts. This is because the linen fibers are predominantly

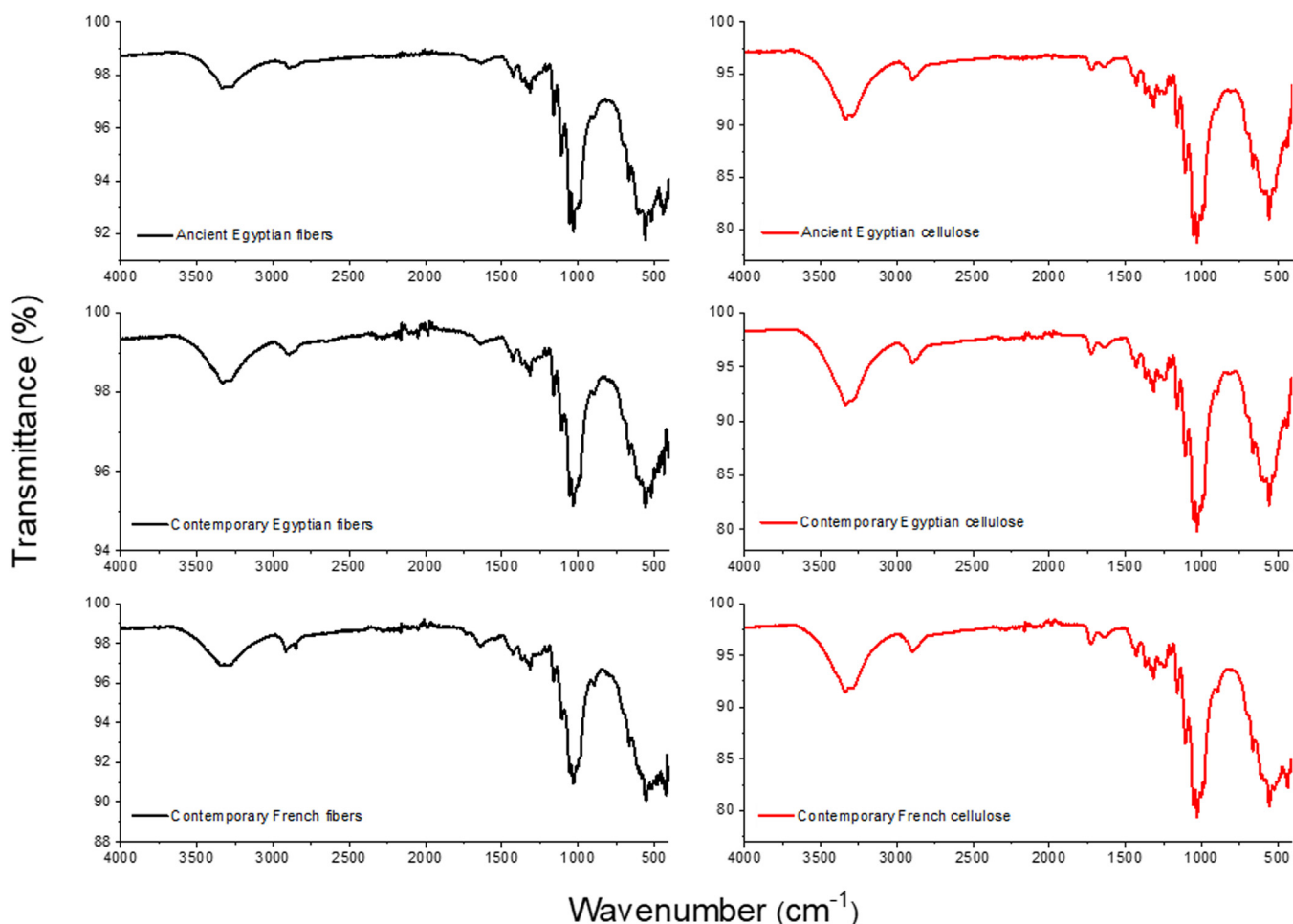


Fig. 4. Comparison between the raw linen fibers and their corresponding cellulose extracts.

composed of cellulose, and therefore, the extraction step does not bring to appreciable differences in $\delta^{13}\text{C}$ values. For the contemporary French samples, $\delta^{13}\text{C}$ mean values of -27.91 ($\sigma = 0.18$; $n = 3$) and -28.47 ($\sigma = 0.13$; $n = 3$), were found for crude fibers and cellulose extract, respectively. In this case, the difference in absolute value of the means is 0.56% with $p = 0.0058$ related to the Student's t-Test, lower than the α value. Although the difference between the means is significant for α value, the experimental uncertainty on the $\delta^{13}\text{C}$ measurements (0.5%) cannot be neglected. Then, the observed differences between the means cannot only be attributed in lignin and hemicellulose content. Secondly, in order to explore the different latitude contribution, a Student's t-Test was performed to compare the $\delta^{13}\text{C}$ values related to the contemporary samples. The comparison of $\delta^{13}\text{C}$ mean values for contemporary Egyptian and French samples, both for raw fibers and cellulose extracts, reveals significant discrepancies. The difference in the mean values obtained from the fibers is 0.35% , while for the extracts it is 0.34% . In both cases, although these differences are statistically significant in agreement with the α value, they should be due to the experimental uncertainty of the technique. Finally, by comparing $\delta^{13}\text{C}$ mean values of the Ancient Egyptian linen samples with those of the Contemporary Egyptian linen, interesting results were obtained. For both raw fibres and cellulose extracts, the means of $\delta^{13}\text{C}$ values were not comparable. The ancient Egyptian flax fibers deviate from the modern one by an absolute value of 3.33% ($p = 2.14 \times 10^{-5}$), while the value of $\delta^{13}\text{C}$ from the cellulose extracts deviate by 3.17% ($p = 3.17 \times 10^{-7}$); re-

sults are schematized in Fig. 2. The differences in the $\delta^{13}\text{C}$ values between the ancient and the modern sample confirms, in part, the impact of the ^{13}C Suess effect on the biological carbon cycle. The ancient Egyptian linen samples show a more positive $\delta^{13}\text{C}$ values compared with the contemporary Egyptian linen samples, reflecting the differences in the atmospheric CO_2 isotopic composition between the XIX Dynasty era (1300–1200 BCE), and the contemporary one. Thus, adjusting as suggested by Dombrosky's work the $\delta^{13}\text{C}$ values of the ancient Egyptian linen samples by adding $+2\%$, new values of $\delta^{13}\text{C}$ comparable with the modern ones were obtained. After the Suess effect correction, we considered the new $\delta^{13}\text{C}$ values coming from the ancient Egyptian linen, which are -26.92% for the raw fibers, and -26.96% for the cellulose extract. However, despite the Suess effect correction, there remains a significant gap between the two samples (Fig. 5). Comparing the $\delta^{13}\text{C}$ values between the historical and the modern Egyptian samples, there is a difference in $\delta^{13}\text{C}$ values of 1.33% for the raw fibers, and 1.14% for the cellulose extracts. As we cannot attribute this discrepancy neither to differences in latitude nor to different photosynthetic pathway of the plant, it is possible to say that, in this case, the effect of fibers degradation causes an isotopic fractionation towards the heavier carbon isotope ^{13}C . Both the raw fibers and the cellulose extract coming from the ancient Egyptian linen sample show more positive $\delta^{13}\text{C}$ compared to the contemporary Egyptians ones, and its means that the material become enriched in the heavier carbon isotope ^{13}C . Similar trends are reported in literature about artificial aging experiments on wood samples [34].

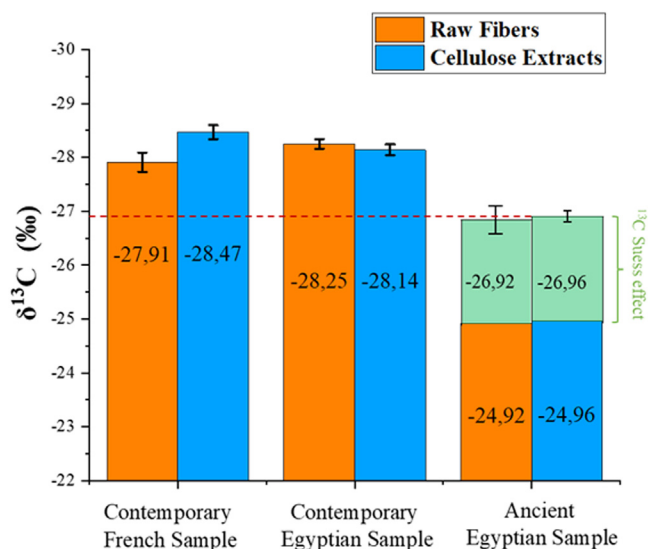


Fig. 5. IRMS response of the raw fibers and cellulose extracts; error bars represent the standard deviation of the measurements. Green histograms represent the gap value coming from the $\delta^{13}\text{C}$ Suess effect.

Conclusion

In this work we studied the degradation process from the perspective of isotopic fractionation of the organic carbon content of ancient Egyptian linen textile fibers. To do this, we compared the $\delta^{13}\text{C}$ mean values of ancient Egyptian linen textile fibers sample with their contemporary Egyptian counterparts, and we found meaningful differences. The $\delta^{13}\text{C}$ moves towards more positive values, ranging from -28.25% , in the case of the Egyptian linen modern sample, to -24.92% in the case of the ancient sample. This discrepancy is in partly due by the $\delta^{13}\text{C}$ Suess Effect; therefore, $\delta^{13}\text{C}$ values of the historical samples were corrected by adding $+2\%$, in order to make comparison with the contemporary samples. There still remain a significant gap. To exclude the possibility that these differences could be due to variables such as different chemical compositions of the fibers in terms of cellulose, hemicellulose and lignin content, pure cellulose was extracted by Brendel method, from all fibers samples examined. In pure cellulose, the same deviation on $\delta^{13}\text{C}$ mean values was observed. Moreover, in order to explore the contribution due to the different geographic latitude of plant growth, $\delta^{13}\text{C}$ value was measured in a sample of modern French linen fibers, and comparison with the $\delta^{13}\text{C}$ value of the modern Egyptian sample, showed no statistically appreciable differences. In conclusion, in this work we have shown that organic carbon during degradation due to natural aging of ancient Egyptian linen textiles fibers is under control of isotopic fractionation in favor of heavier carbon, because the $\delta^{13}\text{C}$ moves towards more positive values over time. However, we believe that further experiments are needed to clarify several aspects concerning the degradation of plant cellulose, especially to give a powerful explanation about the isotopic mechanisms. The degradation of textile samples of historical importance remains an open question in the scientific literature, especially for dating, and for this purpose become crucial understanding whether the isotopic observed fractionation also affects radiocarbon measurements.

Acknowledgement

The authors would like to thank Mr. Rondi Giorgio from "Linificio e Canapificio Nazionale S.r.l." for providing the samples of con-

temporary French linen fibers, and Dr. Viscuso Ezio for SEM images.

This research was supported by "PON03PE_00214_2 DELIAS Project", from University of Catania.

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