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CONFERENCE PAPER

The use of ^{13}C,^{14}C and ^{18}O of dental enamel to estimate the year of birth and geographic origin from Mexican individuals

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Abstract

In recent times, forensic science has increasingly relied on methods that use stable and radioactive isotope analysis to identify human remains. The use of ^{14}C-AMS dating of dental enamel and dentine of an individual allows the estimation of the year of birth, while that of stable isotopes of carbon and oxygen can provide information on their geographical origin. Isotopic analysis of a tissue complements existing identification techniques, enhancing the capacity to refine, exclude, and affirm investigative approaches directed towards individual identification.
The primary aim of this exploratory study is to amalgamate diverse isotopic methodologies conducive to the prompt and accurate identification of a deceased individual. In this manuscript, we elucidate the application of a rapid processing technique for whole molars from individuals with documented ages, employed to ascertain age through ^{14}C-AMS dating. Furthermore, an investigation was undertaken to assess the capacity of carbon and oxygen-stable isotopes in distinguishing regional disparities. To achieve this, we conducted a comparative analysis of tooth samples sourced from individuals residing in three cities within the Mexican Republic: Mexico City in the central region, Oaxaca City in the southern region, and Tepic Nayarit on the western Pacific coast. The age of dental piece formation, as estimated through ^{14}C-AMS, exhibited a precise correlation with the actual age. By means of the stable isotope outcomes, the data disclosed substantial disparities in ^{13}C and ^{18}O abundances among teeth from individuals residing in the three cities.

Introduction

Efficient identification methods in forensic science are essential to providing appropriate humanitarian aid and support to affected communities. In countries like Mexico, where there is a forensic crisis, with thousands of non-identified persons, any information about the physical characteristics of an unidentified deceased person can help to reduce or limit the length of the process to determine their identity. The identification of human remains increasingly employs methods based on the use of stable and radioactive isotopes. Among these, radiocarbon dating allows us to estimate the year of birth or death of individuals. More specifically, the analysis of ^{14}C content in human teeth helps establish the birth times of people who grew up after the 1950s. The technique is based on two factors. The first is the increase of ^{14}C in the atmosphere due to nuclear bomb test detonations since 1955, and then a decrease in ^{14}C after the nuclear test ban treaty in 1963, which produced changes in the concentration of ^{14}C in atmospheric CO_2 (the bomb-pulse) (Hua and Barbetti 2004; Levin and Kromer 2004). The second is that the ^{14}C incorporated in the mineralized tissues remains constant after the tooth is completely formed (Alkass et al. 2011; Cook et al. 2006; Spalding et al. 2005). It is then possible to estimate the formation year of a dental organ or tooth using bomb-pulse dating. Many ^{14}C studies on teeth have shown that tooth enamel has the best fraction for estimating the year of birth with very high precision (Gil-Chavarría et al. 2020; Hodgins 2009; Spalding et al. 2005). Research has indicated that age estimation can be achieved with precision using the entire dental crown, obviating the need for enamel purification (Alkass et al. 2011, 2013).

Stable carbon and oxygen isotopes in human tissues have proven to be valuable in forensic investigations for determining the geographical origin of individuals (Chesson et al. 2020). Carbon isotopic abundance provides insights into an individual's dietary habits, while oxygen isotopes offer information about the water they had consumed.

The stable isotopic concentrations are expressed in terms of δ:

$$\delta_{\text{sample}}(\text{in } ‰) = \left[\left(R_{\text{sample}}/R_{\text{reference}}\right) - 1\right] \times 1000$$

Where R is the atomic ratio between the concentration of the heavy or rare isotope with respect to the light or abundant, $^{13}C/^{12}C$ or $^{18}O/^{16}O$, for the sample and the reference. The isotope ratios $\delta^{13}C$ are expressed in deviations per thousand (‰) from the PDB standard (fossil Belemnite from the Pee Dee formation, and the isotope ratios $\delta^{18}O$ are expressed in deviations per thousand (‰) from the VSMOW standard (Vienna standard ocean water).

Carbon isotopic variation in human tissues reflects the individual's diet of plants and animals, based mainly on two groups of plants, C_3 and C_4: plants with a C_3 photosynthetic pathway (that include trees and main crops), are characterized by $\delta^{13}C$ values ranging from –35 to –20‰ (–26‰ in average); the C_4 plants (that include maize and sugar cane) grow in warm environments and are characterized by $\delta^{13}C$ values ranging from –14 to –10‰ (–12‰ in average) (Bartelink and Chesson 2019). A diet based mainly on C_3 plants will reflect a difference in their $\delta^{13}C$ from those whose diet is based mainly on C_4 plants. Consumers of C_4 plants have higher $\delta^{13}C$ values (~14‰) than consumers of C_3 plants (0 to +2‰) (Kohn and Cerling 2002)

It has been shown that the $\delta^{18}O$ in tooth enamel reflects the isotopic fingerprint of the water consumed during tooth formation and can provide helpful information about the place of geographic provenance and residence (Ammer et al. 2020; Kramer 2018). The geographical distribution of the ^{18}O, the oxygen isotope with the highest mass, is mainly controlled by the environment and climate of a region. Water rich in heavy isotopes remains close to the seas and becomes poorer in ^{18}O as it moves deeper into the continent and also with altitude, resulting in lower $\delta^{18}O$ values (Kramer 2018). Therefore, variations in the ^{18}O ratio allow isotope mapping, making it possible to compare ^{18}O values obtained for teeth from different geographic origins of interest, and to infer whether individuals migrated from one site to another.

Most tooth studies present comparisons between very distant zones (e.g. Alkass et al. 2013); in this work, we explore the potential of carbon and oxygen-stable isotopes to distinguish regional differences. We present ^{14}C, ^{13}C, and ^{18}O data from the dental crowns of individuals who had lived for long time in Mexico, Oaxaca, and Tepic cities, whose year of birth was known. Since different regions can show recognizable dietary isotope differences, we wanted to contrast three cities that are expected to follow different diets: Tepic near the coast in the state of Nayarit, with a seafood-based diet; Oaxaca in the state of Oaxaca, with a traditional Mexican diet, derived from local products; and Mexico City, with a globalized diet derived from a wide variety of products. For the analyses, we implemented a rapid method in the samples preparation protocol to facilitate the processing of many samples (Alkass et al 2011). The year of formation of the dental pieces was experimentally determined from ^{14}C-AMS analysis of the whole crown. The stable isotope analyses were carried out to use ^{13}C and ^{18}O stable isotopes as tracers of Mexico's regional or geographical prints in teeth.

Methodology

The samples were obtained from donors aged 26 years or older through established informed consent in the National Odontological Collection of the National School of Forensic Sciences (ENaCiF-Escuela Nacional de Ciencias Forenses) of the National Autonomous University of Mexico (UNAM-Universidad Nacional Autónoma de México). This project was registered, evaluated, and approved with number FM-DI-045 by the Ethical Board of the School of Medicine of the UNAM. Patients with an indication of extraction necessary for dental treatments (periodontal, orthodontic, prosthesis or surgery) from the Academical Unit of Dentistry of the Autonomous University of Nayarit (UAO-Unidad Académica de Odontología de la Universidad Autónoma de Nayarit) made donation. The set included first (M1), second (M2), and third (M3) molars from individuals of known date of birth, sex, and city of residence. Enamel and dentin formation in molars follow a progressive and differentiated development depending on the tooth. In M1, enamel formation begins at birth and is completed between 2.5 and 3 years, while dentin starts between 3 and 4 months, with root formation concluding around 9 to 10 years. In M2, enamel develops between 2.5 to 3 and 7 to 8 years, with dentin and root formation finalizing between 14 and 16 years (Schour and Massler 1940). The process is more variable in M3, with enamel forming between 7 to 10 and 12 to 16 years, and dentin along with the root completing between 18 and 25 years. Studies have identified statistically significant differences in δ^{13}C values between permanent and deciduous teeth and between permanent teeth formed in early and later stages (Dupras 2007). Archaeological sample analyses indicate that δ^{13}C values vary according to the dental development stage. During breastfeeding, δ^{13}C reflects the isotopic signature of breast milk, typically showing higher values than those observed after weaning, when the introduction of solid foods leads to a new isotopic signature. The informed consent specifies that the mothers of the dental donors were originally from the studied regions and, with one exception (sample LEMA 2093 from Nayarit), resided in those areas during gestation and lactation. The donors continued living in their places of origin.

For this research, teeth from adults were chosen; extractions were performed between 2022 and 2023. The crown was separated from the root using a dental cutting tool, cut in two halves, cleaned in an ultrasonic bath, and then treated in an HCl (0.5M) solution for 10 minutes to remove the outer surface. To estimate the year of teeth formation through the ^{14}C content, a fast preparation process was followed (Alkass et al. 2013). A 200 mg sample of the crown was treated at 70°C using ortho-Phosphoric acid (Merck 85%) to hydrolyze the carbonate into CO_2. This gas was transferred to the carbonate handling system (CHS, Ion Plus), to convert the carbon into graphite (Wacker et al. 2013) and then it was pressed in Al cathodes. The ^{14}C content of the graphite was determined by Accelerator Mass Spectrometry (AMS) at LEMA, Institute of Physics, UNAM on a 1MV Tandetron system (HVEE). The radiocarbon measurements were corrected by fractionation, based on ^{13}C/^{12}C ratios (Solís et al. 2014).

The ^{14}C ages were calibrated using the Northern Hemisphere Zone 2 (NHZ2) bomb radiocarbon data (Hua et al. 2022) and the CaliBomb (http://calib.org) software (Reimer and Reimer 2022).

The ^{14}C-AMS content of molar enamel was used to estimate the year of formation using the method called "bomb-pulse dating" (Alkass et al. 2013). For stable carbon and oxygen isotopes analyses, 50 mg of crown samples were analyzed with isotopic ratio mass spectrometry (IRMS).

Isotopic ratios (δ^{13}C and δ^{18}O) were measured at the Center for Applied Isotope Studies (CAIS), Georgia, with an EA-IRMS 4 Carlo Erba analyzer coupled to a Thermo Delta C, Plus and a 2 Delta V IRMS systems. For ^{13}C the standards were A1296, TSF-1, and NBS 18 with an error $< 0.1‰$. The analyses of δ^{18}O were reported on the VSMOW/SLAP scale, using VSMOW2 and SLAP2 as calibration standards. The combined standard uncertainty is 0.02‰.

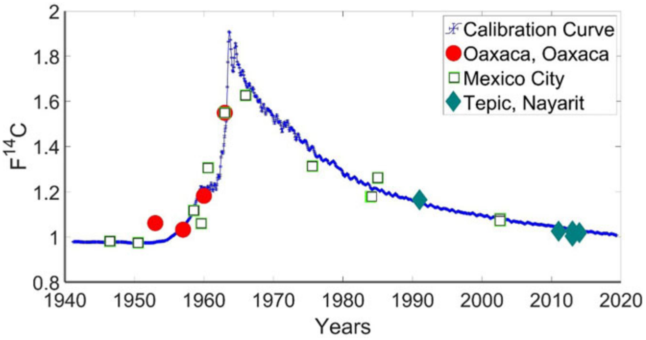

Figure 1. *Fraction of modern carbon ($F^{14}C$) of whole crown of dental pieces from individuals from the cities of Oaxaca, Mexico, and Tepic, plotted onto the atmosphere ^{14}C of CO_2 during the bomb-pulse curve (Hua et al. 2022).*

Results and discussion

The usefulness in forensic science of contemporary teeth (after 1950) comes from their structural and physiological characteristics: enamel and dentin are mainly composed of hydroxyapatite, whose chemical composition is $Ca_{10}(PO_4)6(OH)_2$. De Dios et al. (2015) documented inorganic carbon concentrations of 0.76% in enamel and 0.93% in dentine for human dental specimens. In this work, carbon yield derived from the crowns exhibited a range of 0.32% to 0.57%, with a mean value of 0.50 ± 0.08% w/w. In contrast, to provide a contextual benchmark, tooth enamel extracted exclusively from the crowns of 19 teeth exhibited carbon concentrations spanning from 0.37% to 0.76%, resulting in an average of 0.59 ± 0.10%. This average slightly surpasses the carbon yield attained from the entire crown. Similar carbon yields from isolated enamel and the crown suggest no significant additional carbon contribution from sources other than bioapatite within the dentin.

The carbon yield values obtained align closely with those reported for dental specimens sourced from individuals in Japan (Kunita et al. 2017). The calculation of tooth formation ages using the rapid preparation method shows that the $F^{14}C$ values obtained by analyzing teeth of known age follow the general trend of the bomb-pulse reported for Northern Hemisphere Zone 2 (NHZ2) (Hua et al. 2022) (Figure 1). Calculation of the formation ages gave a difference of –3.4 to 3.0 years with an average of 1.74 ± 1.0 years from the true age. This value is like those reported for teeth from other countries and processes (Alkass et al. 2013; Kunita et al. 2017). The results support the decision to use dental crowns without further treatment to determine the year of tooth formation.

In general, the values of the ages of Mexican teeth fit the curve of the bomb-pulse, except for some cases. A high value of ^{14}C was obtained for an Oaxaca sample, which was from an individual born in 1949. In a previous work in which the year of formation of the same tooth was obtained from collagen and enamel, it was shown that the results for collagen show a slightly greater dispersion in relation to the results for enamel (Gil-Chavarría et al. 2020). In the same study, it was shown that "^{14}C analyses of people born around the 1950s showed ^{14}C inconsistent values, making the estimation of birth year unreliable. This may be the reason for the Oaxaca case since the person was born in 1949." As for the other samples that do not agree with the calibration curve, those of individuals from Mexico City, it is not because the analysis has been done in full crown, but because due to fossil fuel emissions, the air of the Mexico City presents depleted values in ^{14}C that are reflected in the enamel (Flores et al. 2017).

Since Tepic is 40 km from the coast, an impoverishment in ^{14}C values would be expected due to a diet rich in seafood, however, this was not observed. As a matter of fact, marine diets are less likely to affect the ^{14}C content in enamel because carbon in bioapatite is derived from the entire diet and not exclusively from proteins, as in the case of collagen. Moreover, the marine environment absorbed ^{14}C from nuclear testing, which means that marine organisms from the bomb pulse period may not exhibit

Table 1. *Crown enamel samples analyzed for $F^{14}C$, $\delta^{13}C$ and $\delta^{18}O$ with type of tooth, date of birth, and city of residence of the individuals*

Code	Tooth	Birthdate	City of residence	$F^{14}C$	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
LEMA 1942	1st lower molar	02/03/1953	Oaxaca, Oaxaca	1.03	–2.2	–8.7
LEMA 1943	2nd lower molar	02/03/1953	Oaxaca, Oaxaca	1.18	–4.4	–9.3
				1.061		
LEMA 1944	1st lower molar	06/07/1949	Oaxaca, Oaxaca	1.06	–4.2	–10.4
LEMA 1978	2nd lower molar	02/03/1959	Oaxaca, Oaxaca	1.55	–7.7	–9.0
LEMA 1945	1st lower molar	17/05/1962	Mexico City	1.63	–7.6	–8.2
LEMA 1946	1st lower molar	13/03/1975	Mexico City	1.18	–8.5	–7.6
LEMA 1947	2nd lower molar	27/07/1953	Mexico City	1.30	–8.4	–9.6
LEMA 1948	2nd upper molar superior	13/08/1968	Mexico City	1.31	–8.6	–9.3
LEMA 1949	2nd upper molar superior	06/10/1952	Mexico City	1.06	–6.5	–9.5
LEMA 1951	2nd upper molar superior	27/09/1995	Mexico City	1.07	–9.0	–8.3
LEMA 1952	2nd lower molar	27/09/1995	Mexico City	1.08	–8.9	–8.8
LEMA 1979	3rd lower molar	18/06/1968	Mexico City	1.26	–9.6	–7.6
LEMA 2090	3rd lower molar	16/01/2006	Tepic, Nayarit	1.0	–8.5	–5.4
LEMA 2091	3rd lower molar	5/03/1998	Tepic, Nayarit	1.02	–8.6	–5.5
LEMA 2092	3rd upper molar superior	4/10/1997	Tepic, Nayarit	1.03	–9.0	–5.2
LEMA 2093	3rd lower molar	28/09/1995	Tepic, Nayarit	1.16	–9.3	–6.2
LEMA 2094	3rd upper molar	25/09/1975	Tepic, Nayarit	1.03	–8.5	–5.7

Average $\delta^{13}C$ for Oaxaca, Oaxaca (± Std Dev) (in ‰) –4.6 ± 2.3
Average $\delta^{18}O$ for Oaxaca, Oaxaca (± Std Dev) (in ‰) –9.4 ± 0.7
Average $\delta^{13}C$ for Mexico City (± Std Dev) (in ‰) –8.4 ± 1.0
Average $\delta^{18}O$ for Mexico City (± Std Dev) (in ‰) –8.6 ± 0.8
Average $\delta^{13}C$ for Tepic, Nayarit (±Std Dev) (in ‰) –8.8 ± 0.4
Average $\delta^{18}O$ for Tepic, Nayarit City (±Std Dev) (in ‰) –5.6 ± 0.4

significantly older apparent ages compared to terrestrial samples. In the case of the Tepic samples, as they are young individuals, they belong to a period when the difference between atmospheric carbon and that in marine reservoirs is minimal.

The $\delta^{13}C$ and $\delta^{18}O$ values of dental crowns are shown in Table 1. In the $\delta^{13}C$ analysis of teeth, higher values were observed in the teeth of individuals from Oaxaca with average values of –4.6 ± 2‰, followed by those from Mexico City with an average of –8.4 ± 1‰, and finally Tepic with an average of –8.8 ± 0.4‰. The $\delta^{13}C$ of a typical "Mexican supermarket" diet was –21.20 ± 0.3‰. The base material for this diet was made from the menu of the most used foods in Mexico sold in the country's supermarkets. It contains 100 grams of solid diet and 46.8 mL of liquid diet other than water. The resulting mixture was homogenized and freeze-dried. Considering that the isotopic fractionation due to the intake of this diet is 14‰, the value of $\delta^{13}C$ in dental enamel should be around –7‰, an approximate value to those obtained for the teeth of individuals from Mexico City and Tepic (Kohn and Cerling 2002). Meanwhile, the elevated $\delta^{13}C$ values in individuals from Oaxaca suggest a diet primarily based on C_4 plants such as maize. The results of the Tepic teeth indicate a predominantly terrestrial diet and not a marine one, because if so, the $\delta^{13}C$ values should be higher (like a diet based on C_4 plants) (Bartelink and Chesson 2019; Kohn and Cerling 2002). The values of Mexico and Tepic cities, both urban areas, are closer to the values of globalized diets than a city like Oaxaca, with a more Mexican traditional diet based mainly on corn.

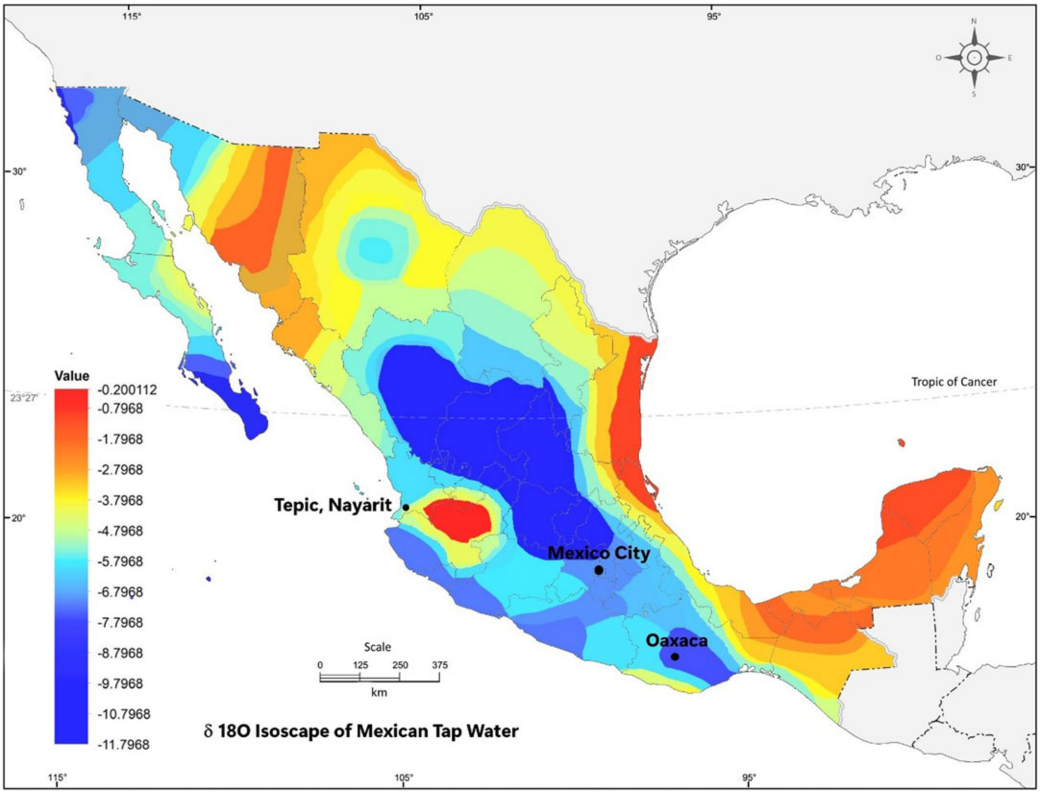

Figure 2. *Map showing the* $\delta^{18}O$ *interpolated isoscape of Mexican tap water with the sampling locations (adapted from Ammer et al. 2020). The black dots show the geographical origin of individuals living in the cities of Tepic, Mexico, and Oaxaca. (Color figure can be viewed at wileyonlinelibrary.com; see Ammer et al. 2020.)*

Oaxaca stood out as distinctly different from other sampling locations, with no overlapping values observed. A pairwise T-test was conducted to assess the differences between $\delta^{13}C$ values in Mexico City and Tepic. The test matched data pairs by the closest dates of birth and used a 0.05 confidence level with 4 degrees of freedom. The calculated test statistic (5.013) exceeded the critical t-value, leading to the rejection of the null hypothesis and indicating that the two groups are statistically different.

The $\delta^{18}O$ values obtained for enamel from whole molars are detailed in Table 1, while a comparative representation with tap water values from Mexico is visually conveyed in Figure 2. In this study, data on the isotopic content of drinking water were used, whose $\delta^{18}O$ values are uniform, as this water is regularly distributed and consumed in the studied region. Isotopic variations due to factors such as evaporation in water treatment plants, boiling water, and the consumption of bottled water should have a minimal impact on the study results. For example, in the case of drinking water in Mexico City, it comes primarily from wells (about 70%) and is filtered and disinfected by chlorination.

The isotopic composition of the teeth showed different ranges of variation. The teeth from Oaxaca varied from –10.4 to –8.7‰ exhibiting an average $\delta^{18}O$ value of –9.4 ± 0.7‰, teeth from Mexico City varied from –7.6 to –9.6‰ with an average value of –8.6 ± 0.8‰, and those from Tepic varied from –5.2 to –6.2‰ with an average value of –5.6 ± 0.4‰. The average values obtained for Oaxaca and Nayarit are within the intervals of the color scale in Figure 2. The only value that does not correspond to the color scale shown in the isoscape, because it is more negative, is that of Mexico City. However, these findings suggest a discernible regional variation in the oxygen isotopic composition of dental tissues, likely reflecting variations in the local isotopic signatures of drinking water.

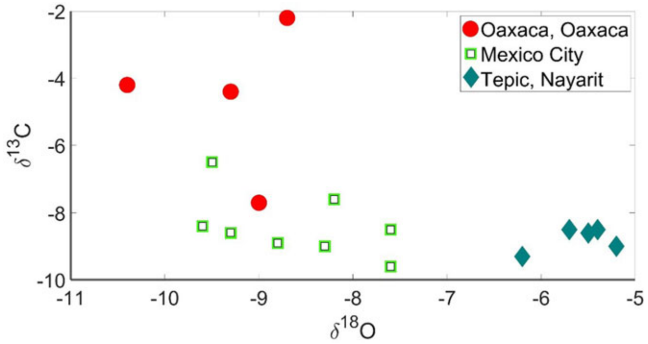

Figure 3. *Bivariate plot of* $\delta^{13}C$ *versus* $\delta^{18}O$ *values for samples from three regions: Oaxaca, Oaxaca (red circles), Mexico City (green squares), and Tepic, Nayarit (blue diamonds).*

A biplot of $\delta^{18}O$ vs. $\delta^{13}C$ is shown in Figure 3 to highlight the differences between locations.

In Figure 3 there is a clustering between the carbon and oxygen isotopic abundances of each zone. There is a separation between the $\delta^{18}O$ values of Tepic and the other two zones. Meanwhile, the values of $\delta^{18}O$ vs. $\delta^{13}C$ allow distinguishing the Oaxaca group from the other two groups. In a well-controlled study, approximately 95% of the data are expected to match the expected result. However, outliers in forensic analyses are inevitable due to individual variations in diet, migration or other external factors. Further analysis and interpretation of these isotopic data could yield insights into factors influencing the observed variations across the studied groups.

Conclusions

The fast method of preparation of dental enamel from individuals of known age was successful because it allowed reconstruction of the age of the teeth.

With the values of $\delta^{13}C$ of the complete crown from individuals residing in three cities in Mexico, it was possible to estimate the range of variation of these isotopes in the three regions. The corresponding statistical results indicate that the differences between the three groups were significant. As well as for $\delta^{13}C$, regarding $\delta^{18}O$ values obtained for whole molars from the three cities, our findings suggest a discernible regional variation in the oxygen isotopic composition of dental tissues, representing an option to delimit the universe in identification processes.

While the current work is exploratory in nature and the sample sizes analyzed from each group are limited, the findings presented in this study are promising in the sense that carbon and oxygen stable isotopes in teeth reflect the provenance of the individuals. We are encouraged by the data and intend to expand our research to include a more substantial sample size.

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