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Reconstructing diet through nitrogen and carbon stable isotope analysis – archaeological and forensic application

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Dissertação de Candidatura ao grau de Mestre em Medicina Legal submetida ao Instituto de Ciências Biomédicas de Abel Salazar da Universidade do Porto.

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Abstract

Stable isotope analysis provides valuable insights into the dietary choices of ancient communities, playing a crucial role in paleodietary reconstructions. Carbon stable isotopes in bone collagen allow us to distinguish between the ingestion of two types of plants (C_3 and C_4), while nitrogen isotopic composition provides an estimation of the amount of protein consumed and the respective trophic level.

In this paper, we analyzed carbon and nitrogen's isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) from human remains recovered from the former 15th-18thcentury St. Theodore's Church in Pula, Croatia. The temporal bone collagen's (n = 31) isotopic results reflect a primarily C_3-plant based diet, with a mild contribution of C_4-plants (such as sugar or millet) and/or marine foodstuff. There were no significant differences in stable isotope values in terms of intra-population variations, such as sex, age, burial type, or period, as far as the samples have provided.

An attempt was made to understand the impact of different customs on the assumed homogeneous monastic dietary habits, and possible sources of isotopic variation are discussed, mainly focusing on the practice of periods of food abstinence, which were especially popular within the community in question. The results were contextualized within known historical and archaeological data.

Overall, these findings have implications in the generic isotopic discrimination of monastic communities, shedding light on potential cultural and geographical isotopic patterns.

In conclusion, this method proves particularly valuable in identifying regional to global patterns evident in various materials, including food products, drugs, and human remains. Consequently, its applicability extends to forensic settings, making it a highly useful tool in such contexts. Therefore, we understand that this method allows archaeological or forensic interpretations of human remains.

Resumo

A análise de isótopos estáveis oferece informações valiosas sobre as escolhas alimentares de comunidades ancestrais, desempenhando um papel crucial nas reconstruções paleodietéticas. Isótopos estáveis de carbono no colagénio ósseo permitem-nos distinguir entre a ingestão de dois tipos de plantas (C_3 e C_4), enquanto a composição isotópica de nitrogénio fornece uma estimativa da quantidade de proteína consumida e do respetivo nível trófico.

Neste artigo, analisámos os *ratios* isotópicos de carbono e nitrogénio ($\delta^{13}C$ e $\delta^{15}N$) de restos humanos recuperados da antiga Igreja de São Teodoro, em Pula, Croácia, datados dos séculos XV a XVII. Os resultados isotópicos do colagénio ósseo dos ossos temporais ($n = 31$) refletem uma dieta principalmente baseada em plantas C_3, com uma leve contribuição de plantas C_4 (como açúcar ou milho) e/ou produtos alimentares marinhos. Não houve diferenças significativas nos valores isotópicos estáveis em termos de variações intra-populacionais, como sexo, idade, tipo de sepultura ou período, conforme os dados fornecidos pelas amostras.

Foi feita uma tentativa de compreender o impacto de diferentes costumes nas supostas práticas alimentares homogéneas da comunidade monástica, e possíveis fontes de variação isotópica foram abordadas, concentrando-nos principalmente na prática de períodos de abstinência alimentar, que eram especialmente populares na comunidade em questão. Os resultados foram contextualizados com base em dados históricos e arqueológicos conhecidos.

No geral, estas descobertas têm então implicações na discriminação isotópica generalizada de comunidades monásticas, dando-nos potenciais padrões isotópicos culturais e geográficos.

Em conclusão, este método demonstra ser particularmente valioso na identificação de padrões regionais a globais, evidentes em vários materiais, incluindo produtos alimentares, drogas e restos humanos. Consequentemente, sua aplicabilidade se estende a ambientes forenses, tornando-se uma ferramenta altamente útil nesses contextos. Portanto, compreendemos que este método permite-nos tanto interpretações de restos humanos arqueológicas como forenses.

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Abbreviations

δ - delta values

δ^{13}C - ratio of ^{13}C to ^{12}C

δ^{15}N – ratio of ^{15}N to ^{14}N

μm - micrometers

AIR – Ambient Inhalable Reservoir

g - grams

M – Molar (mol/L)

m*g* - milligrams

m*L* – milliliters

std – standardized

V - volume

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1. Introduction

1.1. Isotopes

An isotope is a form of a chemical element that has the same number of protons and electrons of that element. While preserving the same chemical properties, it differs on the number of neutrons and therefore has different physical properties (Berto et al., 2018; Schwarcz & Schoeninger, 1991).

The environment we live in is mainly composed by isotopes, which we can divide into two main categories: *radioactive isotopes* and *stable isotopes* (Lederer, 1980). The *radioactive isotopes* have an unstable nucleus, so they suffer a process called radioactive decay, where the isotope spontaneously disintegrates over time by releasing energy (Alves, 2010; Herzog, 2022). The rate in which this disintegration occurs is expressed in half-life (Guillermo & León, 1994), and it is defined by the time it takes for the radioactive element to reach half of its initial activity (Alves, 2010; Guillermo & León, 1994).

On the other hand, the isotopes that don't show any perceptible tendency to change spontaneously, due to its stable nucleus, are considered *stable isotopes* (Ehleringer & Osmond, 1989). Their nuclei tend to be stable when the number of neutrons match the number of protons (Sulzman, 2007), and that is why they don't suffer decay over time. Thanks to this property, stable isotopes analysis is a powerful approach for understanding various physiological processes and interactions with the environment (Ehleringer & Osmond, 1989).

1.2. Collagen

Collagen is the most abundant fibrous protein in the body, with a well-defined amino acid sequence derived manly from dietary protein (Fig.1) (Sobczak-Kupiec et al., 2021). It constitutes around one-quarter of all proteins in mammals and there are at least 15 different types of collagen isomorphs[1] (Schwarcz & Schoeninger, 1991). The most commonly studied is Type I (from this point on, it will be mentioned simply as collagen) and can be found in bones, skin, dentin and tendons (Schwarcz & Schoeninger, 1991). Its main properties are flexibility and traction resistance, and it is the major component of the bone matrix, as well as, one of the only considerable nitrogen sources in bone (Polet & Katzenberg, 2003;

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[1] *chemistry:* molecules with a similar shape

Schwarcz & Schoeninger, 1991). Thus, considering that the isotopic integrity is assessed with easily applicable quality indicators, collagen is one of the first tissues to be used in paleodietary reconstructions (Müldner & Richards, 2005; Polet & Katzenberg, 2003).

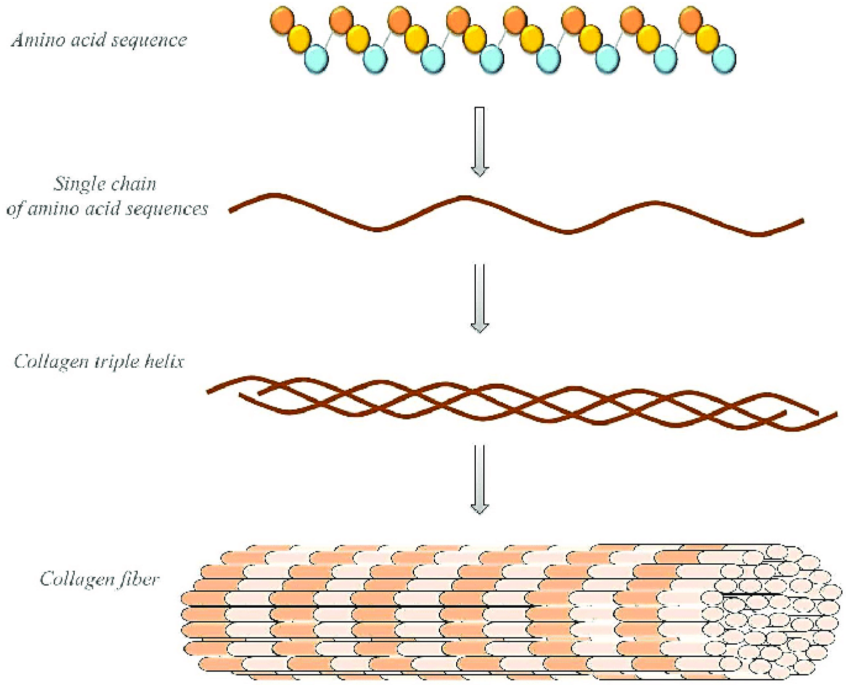

Figure 1 Schematic representation of collagen's structure. Adapted from Sobczak-Kupiec et al., 2021. (p.4)

Throughout the lifetime of an individual the collagen is continuously resorbed and synthetized (Katzenberg & Waters-Rist, 2019). As a consequence of this, collagen is more representative of the main protein diet sources than the diet as a whole (Leatherdale, 2013; Müldner & Richards, 2005). The turnover rate is rather slow, comparing to other body tissues, and it tends to decrease over time, that's why isotopic measurements represent a long-term average of the individual's diet, at least, of the last decade of their life (Chisholm et al., 1982; Müldner & Richards, 2005).

According to Ambrose (1990) and van Klinken (1999), collagen contains around 35-47% of carbon and 11-17% of nitrogen by weight (Katzenberg & Waters-Rist, 2019), subsequently the atom-to-atom ratio is 3:1 of carbon to nitrogen. This ratio is used as a quality indicator, once it denotes if the collagen has undergone diagenesis[2], ranging between 2.9 and 3.6. Anything outside this range is usually excluded by researchers (Ambrose, 1990; DeNiro, 1985).

In view of the fact that collagen has the possibility to degrade over time, depending on postmortem environment, researchers continue to seek other sources representing the lifetime isotopic intake (Katzenberg & Waters-Rist, 2019). For now, collagen is sufficiently well preserved in some human bone remains to permit carrying out stable isotope analysis of dietary reconstructions (Schwarcz & Schoeninger, 2012).

1.3. Stable isotope analysis

Initially stable isotope analysis was focused on geochemical investigations, branching over to ecological studies in the 1970s (Ehleringer & Osmond, 1989). Started off as way to identify the photosynthetic pathway of certain species, but currently it is used for various studies, like nitrogen fixation and water-use efficiency (Ehleringer & Osmond, 1989), as well as reconstructing microenvironments and human ways of living (mobility, nutrition and diseases) in a more archaeological setup.

The stable isotope composition of an organism's tissues is a product of some environmental parameters merged with dietary sources of the elements that are metabolized during its lifetime (Leatherdale, 2013). On this account, and that these elements undergo *isotopic fractionation*[3] during their natural biochemical processes, it's possible to determine dietary behavior by measuring these isotopic differences in the composition of ancient tissue (Leatherdale, 2013; van Der Merwe & Vogel, 1978).

1.3.1. Stable isotope analysis in archaeology

The relative abundance of stable carbon and nitrogen isotopes, are the foundation of paleodietary reconstruction using bone and dentinal collagen (Leatherdale, 2013).This is possible mainly because this method is based in the assumption that the ratio of ^{13}C to ^{12}C (also known as $\delta^{13}C$) and of the ^{15}N to ^{14}N ($\delta^{15}N$) in the collagen corresponds directly to the ratios of the consumers diet, as well as that these ratios vary between food groups (Davies, 2010; DeNiro, 1985).

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[2] postmortem processes that affected the integrity and bone appearance; also known as taphonomy. (Sandford, 1992; Carlson, 1996)

[3] enrichment of one isotope relatively to another in a biochemical reaction. (Urey et al., 2020)

The ratios can be expressed through delta values (δ),

$$\delta^{n}E_{x}= [([^{n}E/^{m}E]_{x}/[^{n}E/^{m}E]_{std})-1]x1000$$

where *E* is the element of the isotope in question for analysis (C or N) and the *n* and *m* correspond to the mass numbers of the less and more abundant isotope respectively (Schwarcz & Schoeninger, 2012). Each sample ratios (x) is compared with their standardized values (*std*) – PeeDee Belemnite (PDB), a marine fossil limestone that was first used as comparison for carbon isotopes ($\delta^{13}C$ set as 0‰), and Ambient Inhalable Reservoir (AIR), referring to atmospheric N_2 (also $\delta^{15}N$ set to 0‰) (Polet & Katzenberg, 2003; Gonfiantini et al., 1993). Since the ratios are small, the unit of $\delta^{n}E_{x}$ is pars per thousand, also known as per mille (‰) (Ambrose, 1987; Chesson et al., 2017; Schwarcz & Schoeninger, 2012). A positive δ points to a prevalence of heavier isotopes in the sample compared to its standard values, while negative values indicate fewer heavy isotopes and more of lighter ones (Sulzman, 2007).

1.3.1.1. Carbon and nitrogen isotopes

Since dietary protein is linked to body protein synthesis, it is known that around 65% of the carbon content in bone collagen is derived from the food intake (Leatherdale, 2013; White, 2003). This carbon is only about 4‰ heavier than in the diet, which is why it is possible to reconstruct the diet from the collagen isotopic composition (Leatherdale, 2013).

In the environment, the isotopes come from two different types of carbon, organic and inorganic. The ratios differ in consonance with the processes involved, varying according to the reaction rates and bond strength. That leads to different fractionation of carbon isotopes relative to their source. (Leatherdale, 2013).

Inorganic carbon is balanced in-between atmospheric carbon dioxide (CO_2), dissolved bicarbonate ion (CHO_3^-) and solid carbonates, hence these exchange reactions, enrich the heaviest isotope's solid carbonate form ($\delta^{13}C$ is 0‰). On the other hand, organic carbon is involved in kinetic reactions of photosynthetic pathways, that increases the concentration of the lighter isotope ($\delta^{13}C$ equals -25‰) (Berto et al., 2018).

Knowing this, the $\delta^{13}C$ measurements help us determine mainly which type of plant was ingested. Terrestrial plants that can be classified as C_3 and C_4, according to their metabolic pathways, have depleted values of $\delta^{13}C$ relative to the atmospheric carbon dioxide values (around -7‰) (Chisholm et al., 1982). Most European plants (typically those from temperate climates) fall into the C_3 category (Davies, 2010) and have an average $\delta^{13}C$ isotopic value of -22‰ to -21‰ in archaeological human bone collagen (Mays, 1997). The C_4 plants, such as maize and sugar canes, are originated in arid climates and its input can be identified with an average ratio in human bone collagen of around -18‰ (Berto et al., 2018; Lightfootet al., 2015; van Der Merwe & Vogel, 1978). Marine plants use both dissolved carbon dioxide and bicarbonate ion resulting in an average $\delta^{13}C$ value of 7.5‰ heavier than terrestrial C_3 plants (Schwarcz & Schoeninger, 1991; White, 2003).

Even though the atmosphere is composed of 78% nitrogen in gaseous form (N_2), the majority of organism are unable to process it (Berto et al., 2018). Nitrogen has to pass through a series of chemical reactions, mainly mediated by microorganisms that fixate it from the soil or from fresh water, so it can finally be consumed, which result in the isotopic fractionation (Berto et al., 2018).

The $\delta^{15}N$ tissue value is used to determine the consumer's trophic levels, but also the consumption of specific plant types (Berto et al., 2018; Leatherdale, 2013). It can help to determine nursing and weaning behaviors in the early stages of life, as well as different kinds of nutritional stress for extended periods of time. Although these reconstructions are mainly made from enamel and dentinal collagen, rather than bone collagen (Leatherdale, 2013).

In plants, the $\delta^{15}N$ values depend primarily on their source of nitrogen – through symbiotic bacterial fixation or directly from the nitrates in the soil (Tykot, 2004). Thus, plants that use bacterially fixed N, have nitrogen ratio values around zero, once it is close to the $\delta^{15}N$ of atmospheric N_2 (0‰) (AIR) (Leatherdale, 2013; Schwarcz & Schoeninger, 2012).

In contrast to carbon, nitrogen isotopes suffer more significant fractionations throughout the food chain (White, 2003). Relative to their dietary source, the $\delta^{15}N$ consumer's amount is enriched by 3 to 5‰ due to fractionation in the metabolism and tissue synthesis (Leatherdale, 2013). Consequently, nitrogen isotopes ratios help to determine the consumers position in the food-web (Polet & Katzenberg, 2003; Sarkic et al., 2019).

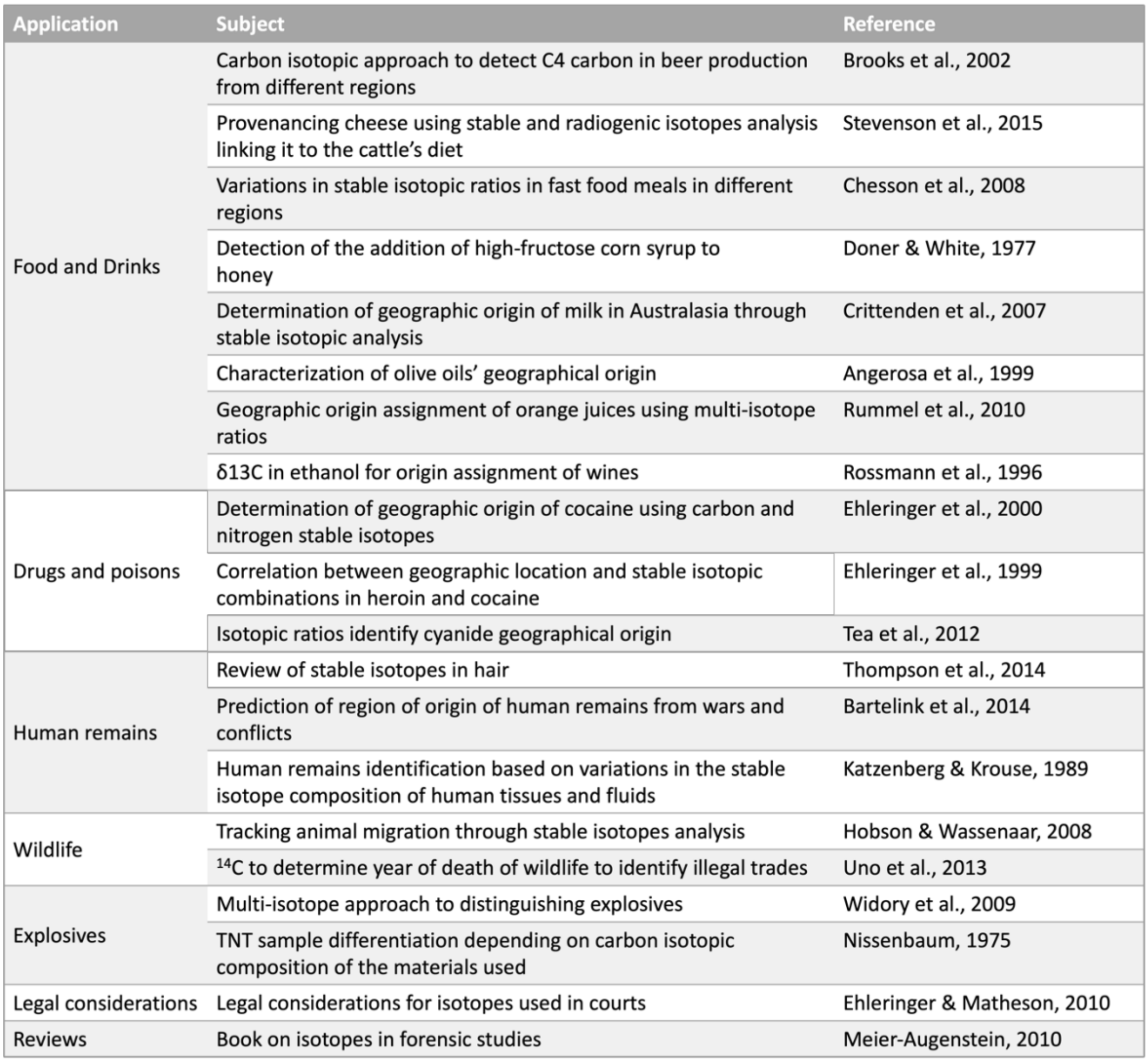
On the account, that most terrestrial food chains have around 3 trophic levels (primary producers, herbivores and finally the predators), as opposed to the trophic levels of marine food chains that can go up to 7 levels, it's clear that marine predators are more abundant and thus have higher $\delta^{15}N$ values than terrestrial ones (White, 2003). That is why nitrogen isotope ratios are better at discriminating between marine or terrestrial ecosystems (Schwarcz & Schoeninger, 1991).

1.3.2. Stable isotopes in forensics

On some other fields, the developments on the analysis of stable isotopes, like archaeology, anthropology, plant physiology, etc., have led some scientists to entertain the possibility that this method can become a tool in the identification of human remains (Chesson et al., 2017). The first studies date back to 1989, by Katzenberg and Krouse, where they discuss the analysis of bone and hair isotopic composition, determining long-term residence in certain areas, as well as the possibility to find if comingling remains point out to the same individual (Chesson et al., 2017; Katzenberg & Krouse, 1989).

From that point on, they tried to continuously expand the database in order to aid forensic investigations (Katzenberg & Krouse, 1989). Nowadays, the available data on human stable isotope ratios is biased towards the areas from which the first studies of comparison appeared (Europe and North America) (Hülsemann et al., 2015). To achieve the best results with this method, other scientists, like Hülsemann (2015), tried to expand the data base to different parts of the world.

Table 1 Representation of the main applications and forensic studies done. Adapted from Cerling et al. (2016).



Application	Subject	Reference
Food and Drinks	Carbon isotopic approach to detect C4 carbon in beer production from different regions	Brooks et al., 2002
	Provenancing cheese using stable and radiogenic isotopes analysis linking it to the cattle's diet	Stevenson et al., 2015
	Variations in stable isotopic ratios in fast food meals in different regions	Chesson et al., 2008
	Detection of the addition of high-fructose corn syrup to honey	Doner & White, 1977
	Determination of geographic origin of milk in Australasia through stable isotopic analysis	Crittenden et al., 2007
	Characterization of olive oils' geographical origin	Angerosa et al., 1999
	Geographic origin assignment of orange juices using multi-isotope ratios	Rummel et al., 2010
	δ13C in ethanol for origin assignment of wines	Rossmann et al., 1996
Drugs and poisons	Determination of geographic origin of cocaine using carbon and nitrogen stable isotopes	Ehleringer et al., 2000
	Correlation between geographic location and stable isotopic combinations in heroin and cocaine	Ehleringer et al., 1999
	Isotopic ratios identify cyanide geographical origin	Tea et al., 2012
Human remains	Review of stable isotopes in hair	Thompson et al., 2014
	Prediction of region of origin of human remains from wars and conflicts	Bartelink et al., 2014
	Human remains identification based on variations in the stable isotope composition of human tissues and fluids	Katzenberg & Krouse, 1989
Wildlife	Tracking animal migration through stable isotopes analysis	Hobson & Wassenaar, 2008
	^{14}C to determine year of death of wildlife to identify illegal trades	Uno et al., 2013
Explosives	Multi-isotope approach to distinguishing explosives	Widory et al., 2009
	TNT sample differentiation depending on carbon isotopic composition of the materials used	Nissenbaum, 1975
Legal considerations	Legal considerations for isotopes used in courts	Ehleringer & Matheson, 2010
Reviews	Book on isotopes in forensic studies	Meier-Augenstein, 2010

Even though this technique isn't a widespread forensic method, it has been increasingly used, not only in multiple cases of human identification, but also in a variety of materials, like counterfeit currency, explosives, illegal drugs, food, wildlife, among others (Chesson et al., 2017; Regan, 2006). Table 1 is a summary of some of these different applications, referencing some studies throughout the time and its findings.

In studies related to food and drinks, the most common subjects are the provenance of products and the detection of "adultered" products (e.g., Doner & White, 1977). The geographic origin through stable isotope analysis is also common in studies about drugs and poisons. The studies about human remains entertains the possibility of this method being used as an investigation tool (Cerling et al., 2016).

Generally, this technique is used when all the other forensic identification methods are exhausted, helping to narrow down the geographical origin of an unknown individual (Chesson et al., 2017; Hülsemann et al., 2015; Rauch et al., 2007). Even though, nowadays a part of the food does not come from local producers, due to international trading, the world's diet data bank gathered so far points to a strong regional distinction, with specific "isotopic-patterns", that can aid profiling the unknown's person identity (Rauch et al., 2007).

To conclude, it is possible that this method can become a routine forensic procedure if certain issues are addressed. To achieve the more accurate results it is still important to expand the database with additional authentic reference data and include data from the analysis of different type of tissues (Rauch et al., 2007).

2. Materials

2.1. About the site

The human osteological samples used on this study represent nuns and rich women who joined the convent community. They were buried in crypts within the St. Theodore Church, located on the Kandler Street (*Kandlerova ulica)*, in Pula, Croatia (Fig.2) (Starac et al., 2011). Pula is a city located in the Istrian peninsula of Croatia. It has a rich cultural and historic heritage, with traces of human settlement tracking back to the prehistoric period.

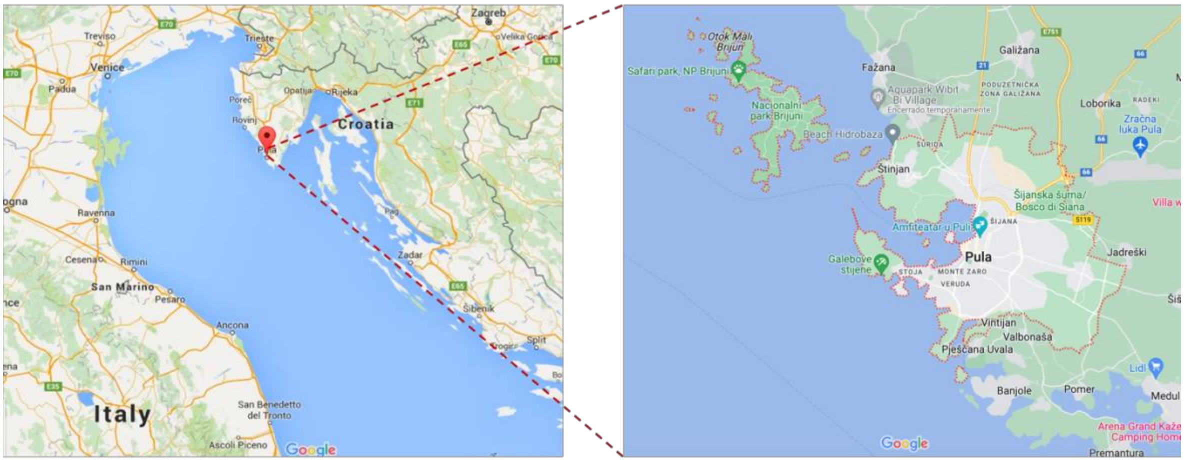


Figure 2 Location of the city of Pula in a map of Europe and its delimitation in the map of Croatia. Google Maps (2023) Pula. Available in https://goo.gl/maps/rmkD1VQBcvqD2Naa6?coh=178573&entry=tt (Accessed: 24 May 2023).

Between the years 2005 and 2009, on the northeastern corner of the city center of Pula, archaeological research led by the Archaeological Museum of Istria, rediscovered an interesting heritage of the city. Throughout those years, the researchers found buildings remains and movable tangible cultural assets, dated from the beginning of the 1st millennium BC to the 20th century AD. The closest one to the surface, and more recent, was the complex relative to the women's Benedictine monastery, where it can be found the church of St. Theodore (Fig.3) (Krnjak, 2010). Historical sources state that this church was erected in 1458, alongside the ruins of a previous church, St. Lucia's Church. It was abandoned in the 18th century and demolished after that in the 19th century for military purposes (Krnjak, 2010).

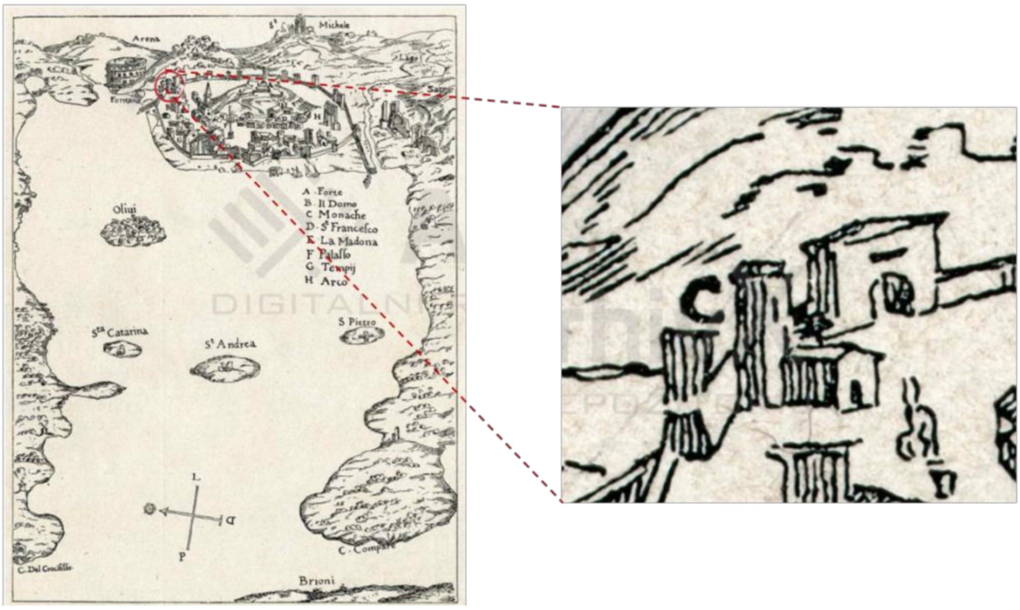


Figure 3 Diagram representing the view of the city of Pula in Descriptio portus et urbis Polae di A. de Ville, 1633 with a zoom on a depicting sketch of the church of S. Teodoro (source: Krnjak, 2010, p.7) –

The floor of said church wasn't preserved, but below it lays 18 burial crypts that were walled in and covered with stone slabs and metal rings (Fig.4). Within each crypt there was a considerable number of skeletal burials. It was documented that around one hundred interred individuals, most of which were buried in garments embroidered with floral decorations and equipped with small rings, crowns and rosaries, small metal medallions or crucifixes (Krnjak, 2010; Starac et al., 2011).

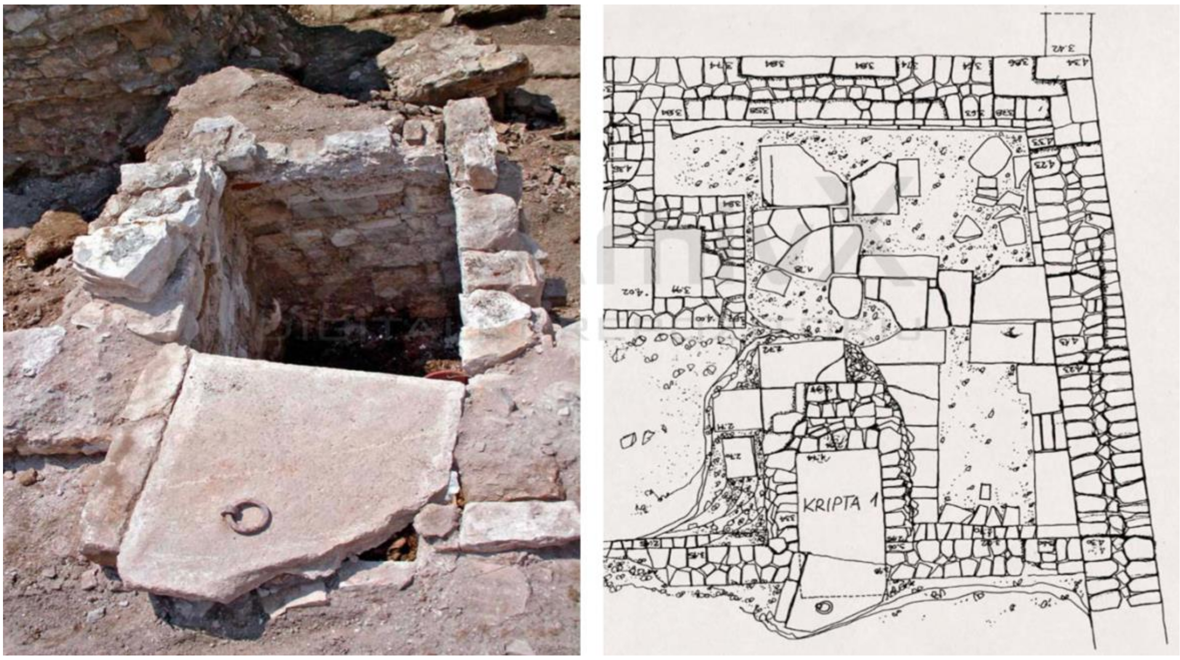


Figure 4 Crypt number 1 from the St. Theodore's Church. Photograph (right) by Dr. Sc. A. Starac and sketch of the location of the crypt (left) by S.Bertoldi. Adapted from "SVETAČKE MEDALJICE: pobožna znamenja žiteljica samostana Sv. Teodora u Puli", by O. Krnjak, 2010, Catalogue from Archaeological Museum of Istria, Pula (81), p. 15. Copyright 2010 by "ARHEOLOŠKI MUZEJ ISTRE – PULA".

2.2. Human skeletal remains from the site

The human skeletal remains found were analyzed to gather data on health and diseases of ancient inhabitants of Pula. However, since the majority of the graves were commingled and with incomplete remains, the analysis and interpretation of data was limited. For each of the 18 unearthed graves, it was established a minimal number of individuals. When it was possible to individualize a skeleton, the sex, age and pathological changes were established (Starac et al., 2011).

Figure 5 Photograph of grave number 10, located on the west side of the St. Theodore's Church. Adapted from "PULA: The Birth of a Town", by A. Starac, 2011, Catalogue from Archaeological Museum of Istria, Pula (83), p.60. Copyright 2011 by the Archaeological Museum of Istria.

Figure 6 Photograph of crypt number 9 as an example of the appearance of an unearthed crypt in St.Theodore's Church, from "PULA: The Birth of a Town", by A. Starac, 2011, Catalogue from Archaeological Museum of Istria, Pula (83), p.60. Copyright 2011 by the Archaeological Museum of Istria.

At this burial site, a minimal number of individuals was narrowed down to 71, out of which 57 was established to be adults and 14 subadults (most of whom were adolescents). Out of the 57 adults it was only possible to establish the sex of 32 individuals, due to poor state of preservation of the bone elements. There were 28 females and 4 males, which confirmed the assumption, in accordance with historical sources, that the nuns and wealthy women that lived at the convent were buried within the church. The age-at-death of the females was between 20 and 34 years whereas the males were all over the age of 35. Several cases of pathological changes were recorded, like scurvy and tuberculosis, as well as bone fractures, diffuse idiopathic skeletal hyperostosis and a few cases of degenerative changes on long bones and vertebrae (Starac et al., 2011).

2.3. Samples

All 71 samples of temporal bones acquired from the human remains that had been unearthed from the crypts of Kandlerova and were analyzed at the Institute for Anthropological Research in Zagreb, where a total of 31 samples have been taken into account for this dissertation (*n*=31).

Table 2 Samples used in the research, according to crypt and grave number.

Site	Crypt Number	Grave Number	Sample name
Pula Kandlerova	1		P-K1-A
			P-K1-B
			P-K1-C
			P-K1-D
			P-K1-E
			P-K1-F
			P-K1-G
	3-5	GRAVE 1	P-K3-3-G1
		GRAVE 2	P-K3-5-G2
		GRAVE 3	P-K3-5-G3
		GRAVE 7	P-K3-5-G7
		GRAVE 9	P-K3-5-G9
		GRAVE 10	P-K3-5-G10
	7		P-K7-A
			P-K7-B
			P-K7-C
	10		P-K10-A
			P-K10-B
			P-K10-C
			P-K10-D
			P-K10-E
			P-K10-F
			P-K10-G
			P-K10-H
			P-K10-I
			P-K10-J
			P-K10-K
			P-K10-L
			P-K10-M
			P-K10-N
			P-K10-O

3. Methods

3.1. Cleaning by abrasion

All the 31 samples of temporal bone's fragments were cut into smaller pieces, not lighter than 0,3g, with a Dremel diamond-coated circular cutting wheel, so they can be placed in cylindrical test tubes (seen in Fig.7). Subsequently, their surface was abraded with a sandpaper burr, so the fragments were cleansed from any contaminants, in order to fasten further procedures.

All drill bits were cleaned prior to their use, and in between each sample using 70% ethanol solution V/V, to ensure that there is no cross contamination between sample powder.

3.2. Demineralization in HCl

Before soaking the samples, each of the final cuts of the sample was weighted (mg) in an analytical balance in order to have a precise starting mass of the samples. (Tab. 3).

Table 3 Start mass of samples in milligrams (mg).

Sample Name	Start Mass (mg)
P-K1-A	897.5
P-K1-B	682.8
P-K1-C	701.5
P-K1-D	1114.3
P-K1-E	571.5
P-K1-F	742.3
P-K1-G	680.6
P-K3-5-G1	813.8
P-K3-5-G2	1182.4
P-K3-5-G3	1446.8
P-K3-5-G7	783.2
P-K3-5-G9	1102.3
P-K3-5-G10	576.1
P-K7-A	1171.6
P-K7-B	1145.8
P-K7-C	904.5
P-K10-A	911.0
P-K10-B	900.9
P-K10-C	959.4
P-K10-D	1338.2
P-K10-E	968.7
P-K10-F	896.6
P-K10-G	809.7
P-K10-H	1010.7
P-K10-I	1011.1
P-K10-J	1045.7
P-K10-K	1409.1
P-K10-L	1460.2
P-K10-M	855.3
P-K10-N	1276.1
P-K10-O	1288.8

All bone samples were immersed in 0.5M HCl (Fig.7), held at 4ºC for several days, until the minerals around the collagen dissolved. As the demineralization advanced, daily checks were performed and the hydrochloric acid (HCl) was changed every 3 days to optimize the process of demineralization, rinsing the samples with deionized water every time.

Figure 7 Example of test tube with bone cut soaked in HCl (0.5 M).

Figure 8 Samples placed in the oven (Thermo Scientific™ dry sterilizer)

After the full demineralization, the samples were rinsed three times in deionized water and then each tube was fully filled with deionized water (approximately 13mL) adding three drops of 0.5 M HCl to achieve a pH of 3 and sealed properly afterwards. Next, they were placed in the oven (Thermo Scientific™ dry sterilizer) at 80ºC for 48h (Fig.8) to gelatinize the protein.

3.3. Collagen extraction

Consecutively, E-zee filtration was performed to remove >80 µm particles (insoluble residues) from the gelatinized protein (Fig. 9). The final liquid solution from each sample was separated into three different test tubes with a little bit more than 4mL in each, as observable in the Fig.9. All the samples were placed in the freezer for 24h and then freeze-dried for 48h (Fig.10).

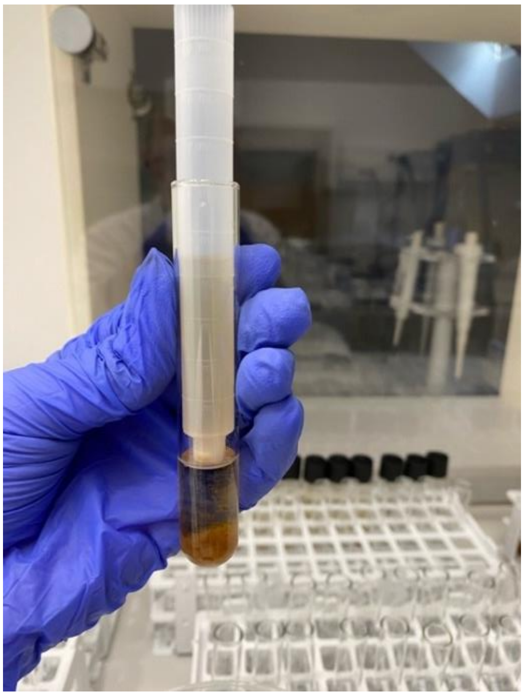


Figure 9 Example of the E-zee filtration of 4mL of the final liquid solution containing the gelatinized protein.

Figure 10 Samples placed in the freeze-dryer (HyperCOOL HC3055).

Lastly, the collagen extracted from each bone sample was weighted and inserted in Eppendorf 1,5 mL FlexTubes®.

3.4. Mass spectrometry

The samples of collagen extracted from the bone cuts were sent to the Iso Analytical Laboratory in Crewe, UK, where they were loaded into an auto sampler Europa Scientific Roboprep-CN sample preparation module.

Stable isotope ratios of N and C were measured via Elemental Analysis of Isotope Ratio Mass Spectrometry (EA-IRMS) (Berto et al., 2018; Ehleringer & Osmond, 1989). It separates ions according to their mass-charge ratios and measures their values relatively to the standard reference of each molecule (in this case study, the Pee Dee Belemnite carbonate, for C, and atmospheric nitrogen, for N) (Berto et al., 2018; Polet & Katzenberg, 2003).

4. Results and Discussion

As Ambrose (1990) stated, well preserved collagen has final carbon yields of >13% and nitrogen yields of >4.8%, and needs to be within the 2.9-3.6 C:N ratio range (Privat et al., 2002). As we can observe in Table 4, all the samples of collagen met all the reliability indicators (%C, %N and C:N ratio range), with all being considered as valid. Stable carbon and nitrogen measurements are presented in Table 4 for each bone element.

Table 4 Stable carbon and nitrogen isotopic results for the individuals of Pula Kandlerova and reliability indicators of each sample.

Samples	Bone Element	Carbon Content (%)	$\delta^{13}C$ (‰)	Mean $\delta^{13}C$ (‰)	1σ (C)	Nitrogen Content (%)	$\delta^{15}N$ (‰)	Mean $\delta^{15}N$ (‰)	1σ (N)	C:N
P-K1-A	Temporal	41.3	-19.1	-18.8	0.3	15.5	11.2	10.3	0.7	3.1
P-K1-B	Temporal	44.2	-18.3			16.6	10.9			3.1
P-K1-C	Temporal	44.1	-18.9			16.5	10.9			3.1
P-K1-D	Temporal	44.1	-19.3			16.8	9.9			3.1
P-K1-E	Temporal	43.2	-18.5			16.2	10.0			3.1
P-K1-F	Temporal	43.7	-19.0			16.2	10.4			3.1
P-K1-G	Temporal	44.2	-18.9			16.6	10.1			3.1
P-K3-5-G1	Temporal	35.3	-19.2			13.0	10.2			3.2
P-K3-5-G2	Temporal	36.5	-19.2			13.5	10.3			3.2
P-K3-5-G3	Temporal	44.5	-18.1			16.6	10.0			3.1
P-K3-5-G7	Temporal	42.2	-18.8			15.6	8.8			3.2
P-K3-5-G9	Temporal	44.1	-19.1			16.3	9.9			3.2
P-K3-5-G10	Temporal	43.4	-19.1			16.0	9.1			3.2
P-K7-A	Temporal	44.1	-18.2			16.3	11.3			3.2
P-K7-B	Temporal	43.9	-18.7			16.1	10.6			3.2
P-K7-C	Temporal	44.0	-19.0			16.2	10.4			3.2
P-K10-A	Temporal	44.3	-18.5			16.2	10.3			3.2
P-K10-B	Temporal	43.8	-18.6			16.0	10.1			3.2
P-K10-C	Temporal	43.5	-19.2			15.8	10.7			3.2
P-K10-D	Temporal	44.9	-18.7			16.4	10.0			3.2
P-K10-E	Temporal	42.9	-18.4			15.9	10.3			3.1
P-K10-F	Temporal	42.7	-18.9			16.0	9.4			3.1
P-K10-G	Temporal	42.8	-18.4			15.7	9.4			3.2
P-K10-H	Temporal	39.6	-19.2			14.8	10.5			3.1
P-K10-I	Temporal	42.5	-18.6			15.9	11.2			3.1
P-K10-J	Temporal	42.4	-19.3			15.6	11.0			3.2
P-K10-K	Temporal	43.2	-18.9			15.9	10.8			3.2
P-K10-L	Temporal	43.6	-19.0			16.1	10.3			3.2
P-K10-M	Temporal	41.1	-18.6			15.2	9.6			3.2
P-K10-N	Temporal	42.6	-18.5			15.9	10.8			3.1
P-K10-O	Temporal	43.0	-18.6			15.9	11.8			3.2

The δ^{13}C values range between -19.3 and -18.1‰ with a mean value of -18.8‰±0.3 and δ^{15}N between 8.8 and 11.8‰, with a mean value of 10.3‰±0.7.

The values of δ^{13}C and δ^{15}N between individuals don't show significant variations (Fig.11) which means that the nuns held a homogenous diet. The δ^{15}N may present a bigger difference between the highest value of 11.8‰ and the lowest of 8.8‰, which can mean there was a slight variation in animal protein intake, possibly explained by personal preferences, individual physiological differences on the basal metabolic rate, or the effect of nitrogen imbalance due to fasting, as described in the literature (Fuller et al., 2005; Katzenberg & Lovell, 1999; Sarkic et al., 2019).

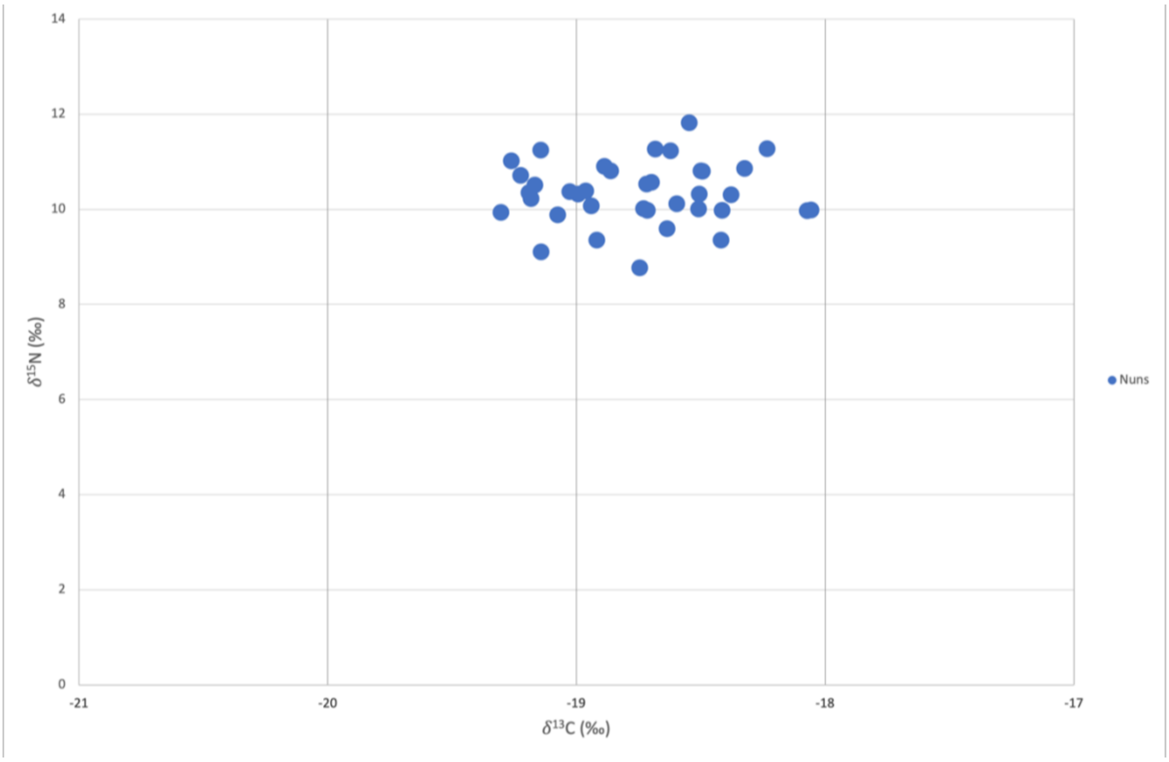


Figure 11 Human collagen δ^{13}C and δ^{15}N isotopic values for the nuns of St. Theodore's Church in Pula Kandlerova

Since virtually identical, it was not possible to differentiate the results based on sex or status. It was not possible to determine the age of the individuals, a factor which has therefore not been included in further interpretation.

The isotopic data demonstrates that the nuns had a C$_3$ based diet, consisting of local food webs and autochthonous plants. Given the church's location (Fig. 2 and 3, from the previous chapter) marine food intake can't be ruled out with δ^{13}C and δ^{15}N profiles such as these, that usually indicate some marine protein consumption on a daily basis. With $\delta^{13}C$ values <-18‰, it is assumed that there was a certain C_4 intake rather than extensive consumption of marine foodstuff. Had that been the case, the $\delta^{15}N$ values would be more positive.

Archaeobotanical and zooarchaeological studies from Croatia are limited, due to the fact that these are quite recent disciplines in Croatian archaeology (Lightfoot et al., 2012). Nonetheless, studies done by Becker (2001) and Chapman (1996) track the appearance of broomcorn and foxtrail millet on coastal and inland Croatia around the Bronze and Iron Age (Filipović et al., 2020; Lightfoot, et al., 2015). There are also studies that show environmental climate changes, from colder to warmer, over the Roman to Early Medieval transition (e.g., McDermott et al., 2001), so it is possible to speculate, that there was a substantial increase in the consumption of millet in Croatia from that point on, once it is a warm temperature and drought-tolerant cereal (Lightfoot et al., 2012).

There are studies about stable isotopic ratios that differentiate female diet from male diet, done in Roman and Pre-Roman populations, where females appear to consume higher amounts of cereals in general (C_3 or C_4 based plants), while males have greater access to meat or fish (Alaica et al., 2019; Laffranchi et al., 2016). This difference might be attributed to cultural restrictions based on perceived negative effects on female health (Alaica et al., 2019). Even though this fact might give an insight to the type of diet that the nuns had, in the current study, this hypothesis can't be corroborated because it is a fully female population.

At last, these conclusions may suggest that the type of C_4 plant more likely to be responsible for the enriched $\delta^{13}C$ values is mainly the millet, which was a crop easily accessible to people of all social statuses. Without a proper faunal isotopic analysis of zooarchaeological samples from this site, it's impossible to know if the presumed millet was consumed directly by humans, or the $\delta^{13}C$ values are associated with a significant amount of C_4 plants from animal's diet (Lightfoot et al., 2015).

In order to further understand the results, it is important to compare this region's results to others in Croatia from different time periods (Table 5). Lightfoot's *et al.* (2012 and 2015) data, from various sites throughout different periods (Bronze Age, Iron Age, Roman and Early Medieval) show populations whose diet mainly consistent of C_3 terrestrial resources, with some C_4 plants or marine foodstuff intake (Lightfoot et al., 2012; 2015). Though some statistical differences are visible, they were explained by major cultural changes from one period to another. As an example, the transition from the Roman to Early Medieval period displays a clear abandonment of marine resources by some populations (Table 5 and Fig. 12), attributed to the conscious elimination of Roman dietary habits (Lightfoot et al., 2012). Therefore, apart from the Early Medieval data which presented higher δ^{13}C values, most results from coastal regions (Table 5) resemble the ones obtained in the current study.

Table 5 δ^{13}C and δ^{15}N mean values from various sites of Croatia divided by time period. Data adapted from Lightfoot et al., 2012; 2015

Time Period	Location	Mean δ^{13}C (‰)	Mean δ^{15}N (‰)	References
Bronze Age	Coastal (Nadin-Gradina)	-18,5	9,6	(Lightfoot et al.,2015)
	Inland	-19,9	10,8	
Iron Age	Coastal (Nadin-Gradina)	-18,8	9,7	(Lightfoot et al., 2012, 2015)
	Inland	-18,4	9,1	(Lightfoot et al.,2015)
Roman Age	Coastal/Island (Zadar-Relje/Vis-Bandirica)	-18,8	10,1	(Lightfoot et al.,2012)
Early Medieval	Coastal	-17,9	9,6	(Lightfoot et al.,2012)

The samples from both Bronze and Iron Age coastal individuals were retrieved from the same site of Nadin-Gradina, and don't show significant differences between the two time periods. As we can observe in Table 5, the Roman Age δ^{13}C and δ^{15}N values don't differ much from the Iron Age ones as well. So, these profiles suggest that the individuals had predominantly C_3-based diets with some contribution of animal protein and/or millet (Lightfoot et al., 2012; 2015). The fact that for inland populations, the δ^{13}C and δ^{15}N values don't appear to have significant differences from the coastal ones, leads us to believe that, in both populations, the C_4 element/marine products had the same weight in the C_3-based diet and that the easy access to certain type of foods did not influence the dietary habits (Lightfoot et al., 2015). Because carbon values of a marine diet and a C_4-plant based diet tend to overlap (Alaica et al., 2019; Miller, 2018; Schoeninger et al., 1983), it is challenging to determine which one of these elements had an input in this populations' diets. However, considering that the δ^{15}N values are not significantly elevated to represent marine food consumption, it is more plausible that there was a slight consumption of C_4 plants (Miller, 2018; Reitsema, 2013).

Aside from these comparison data, Lighfoot's *et al.* (2012) work provides insights into the baseline isotopic values of coastal regions in Croatia during the Early Medieval Period (EMP – Fig. 12), examining a range of fauna from herbivores to carnivores. Connecting those results to faunal isotopic levels presented at Dreshaj's (2017) thesis on the Late Medieval Period (LMP – Fig. 12) in Bribir, we can establish an isotopic food web that serves as reference point for interpreting the dietary habits of the nuns of Pula Kandlerova. The isotopic values of coastal individuals from the Early Medieval period were also integrated in Fig. 12, for a better understanding of the shift away from Roman dietary practices discussed earlier (Table 5) (Lightfoot et al., 2012).

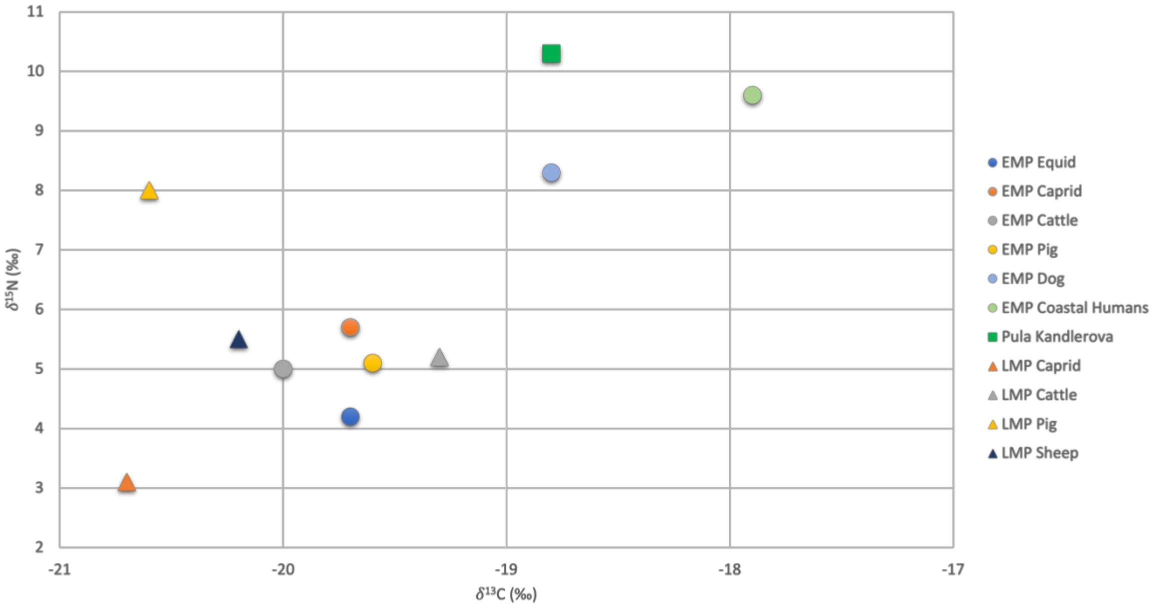


Figure 12 Faunal bone collagen isotopic results from Early Medieval Period and Late Medieval Period, separated by species. Comparative $\delta^{13}C$ and $\delta^{15}N$ values of a human population from the coastal side of Croatia and Pula Kandlerova. Adapted from data in Dreshaj, 2017; Lightfoot et al., 2012.

While faunal values might be misleading as they are not site nor time specific (Miller, 2018), it is worth noting that the isotopic values of the Pula Kandlerova nuns are significantly enriched compared to these faunae, indicating that they cannot be solely attributed to the consumption of C_3 fruits and vegetables. It supports the hypothesis of a mixed protein-carbohydrate diet, potentially including C_4 sources and protein from sea-based sources such as fish and its derivates. The enriched $\delta^{15}N$ can also be explained by high environmental nitrogen baseline values or, as mentioned earlier, by metabolic changes caused from fasting in compliance with monastic rules.

In monastic communities, it is known that the diet follows the Benedictine laws, which basically proscribes the eating of meat at specific days of the week, or in overall fasting seasons (Mays, 1997; Simčenka et al., 2020). Only the sick would be excused of this monastic rule, going back to fasting as soon as their health was restored. Thus, the monastic diet is expected to consist mainly in terrestrial C_3 plants, with a significant contribution of seafood products that could substitute terrestrial animal protein (Mays, 1997; Polet & Katzenberg, 2003; Simčenka et al., 2020).

In fact, the stable isotopic analysis of human remains from some monastic communities around Europe, in countries like Belgium, England, Lithuania (e.g.: Polet & Katzenberg, 2003; Quintelier et al., 2014; Sarkic et al., 2019; Simčenka et al., 2020; Yoder, 2012), validates that their diet relied heavily on terrestrial food resources (C_3 plants like rye, barley, wheat and other cereals) with appropriate marine intake (Müldner & Richards, 2005; Simčenka et al., 2020). Still, this intake proportionally led to less negative $\delta^{13}C$ levels in coastal sites than those further inland, consequently indicating that the isotopic values rather reflect the ease of access to marine resources, than a consequence of the monastic diet rules (Mays, 1997).

4.1. Iberian Peninsula

Isotopic studies such as this have shown the importance of successfully estimating relative contribution of marine foodstuff versus C_3-based terrestrial resources in coastal populations, since it is possible to identify distinctive isotopic signatures in these ecosystems, which in turn allows for generic discriminations of these regions (Cubas et al., 2019).

Table 6 Iberian peninsula's $\delta^{13}C$ and $\delta^{15}N$ isotopic values divided by period and area. Based on data from Alaica et al., 2019; Carvalho & Petchey, 2013; Cubas et al., 2019; Sarkic et al., 2019

Period	Location		Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)	References
Mesolithic	Atlantic		-18 ± 1.4	10.7 ± 1.6	(Cubas et al., 2019)
	Mediterranean		-18.4 ± 0.5	9.5 ± 1.2	
Neolithic	Atlantic		-19.6 ± 1.0	8.9 ± 1.8	
	Portugal	São Paulo	-18.10	10.0	(Carvalho & Petchey, 2013)
		Castelo Belinho	-18.02	10.86	
	Mediterranean		-19 ± 0.6	9.3 ± 1.5	(Cubas et al., 2019)
Late Roman Era Early Middle Age (Early Byzantine)	Mediterranean		-18.07 ± 0.5	10.1 ± 1.3	(Alaica et al., 2019)
	Spain	Ibiza			
Modern Age (16th-17th century)	Spain	Belmonte	-18.0 ± 0.4	11.4 ± 0.8	(Sarkic et al., 2019)

So, comparing the results in this study with others, it is possible to verify that they share similar results to studies on Atlantic and Mediterranean coast populations in the Iberian Peninsula (Table 6). The data suggested the consumption of essentially C_3-terrestrial foods by these populations on both sides of the Peninsula, with as much as 23% contribution of marine protein on the Atlantic zone, where the access to the resources were facilitated by the higher productivity and the larger tidal zones compared to the Mediterranean (Alaica et al., 2019; Carvalho & Petchey, 2013; Cubas et al., 2019).

It is also possible to track the dietary changes associated with the introduction, expansion, and establishment of a farming economy in these coastal regions throughout time (Cubas et al., 2019). A substantial switch from the consumption of marine foods is noticeable through the $\delta^{15}N$ values of the Atlantic region in the Early Neolithic (Table 6), which is linked to the onset of farming in the Coastal Peninsula (Cubas et al., 2019; Guiry et al., 2016). A more heterogeneous diet of various resources (terrestrial animals, wild terrestrial plants, marine foodstuff, etc.) was replaced by a farming product (crops and livestock), where it was possible to control the production cycles (Cubas et al., 2019).

Although it seems counterintuitive, especially in coastal regions, or even in islands like Ibiza (Alaica et al., 2019), a strong dependence on terrestrial resources in the Atlantic population has been noted, linked to ideological changes in how food is valued (Waterman et al., 2016). In the same way, the Mediterranean populations' diet consisted mostly of cereals like wheat, barley, rye and oats, as well as meat, animal products, fish, and/or derived products, depending on the social status (Alaica et al., 2019; Cubas et al., 2019).

Archaeobotanical data from Iberian Peninsula tracks the beginning of the incorporation of C_4 plants (millet) in the dietary habits back to the Middle Bronze Age (Alaica et al., 2019). In a Mediterranean population (Joan Planells, Ibiza) of the Late Roman-Early Byzantine, the $\delta^{13}C$ and $\delta^{15}N$ mean values that rounded − 18.7 ± 0.5 and 10.1 ± 1.3‰, respectively (Table 6), are already associated to C_4 plants consumption, rather than marine protein ingestion (Alaica et al., 2019). However, it is important to note that the possibility of some marine protein ingestion is not completely ruled out (Alaica et al., 2019; Quintelier et al., 2014).

In the Atlantic region, mainly Portugal, the spread of millet came a little bit later, although there are some Neolithic populations that show values in keep with a C_4 plant incorporation diet [for example: São Paulo ($\delta^{15}N$= 10.0, $\delta^{13}C$= −18.10); Castelo Belinho ($\delta^{15}N$= 10.86, $\delta^{13}C$= −18.02; 20% marine carbon (Table6)) (Carvalho & Petchey, 2013)]. The enriched $\delta^{13}C$ values might suggest an early introduction of millet into Portugal (Waterman et al., 2016), or the consumption of seaweeds, known to be a C_4 plant, and its usage as crops' fertilizer (Waterman et al., 2016).

Finally, it is important to compare the results from this research to an equivalent study population. Sarkic et al. (2019) reconstructed the diet of a 16th-17th century community from the convent of Santa Catalina de Siena, in Belmonte, Spain (Table 6). This convent nuns' isotopic ratios suggest the same compliance to the monastic rules as this study's population, where the $\delta^{15}N$ values, which Sarkic et al. (2019) compared to that site's isotopic faunal baseline, were indicative of a low terrestrial animal protein consumption. The diet was strict and mainly based in C_3 vegetables, but with a slightly more detectable input of marine foodstuff (Sarkic et al., 2019), compared to this research's results. These conclusions allow a generic discrimination of these communities.

5. Conclusion

The application of stable isotope analysis in the archaeological set up that we encounter in this research allows us to better understand dietary patterns of Croatia's coastal communities, more specifically monastic individuals.

In this thesis it was carried out a dietary reconstruction of a monastic female population through isotopic data analysis extracted from bone collagen. The results allowed to identify aspects of uniformity amongst members, with no negative evidence—in terms of malnourishment —registered in the bones from the diet restrictions imposed by their order's rules.

Overall, the results suggest a primarily C_3-plant based diet, fitting with the historical evidence of cereals and vegetables' availability around this region. Additionally, it is possible to identify a certain contribution of C_4-plants (sugar or millet) and/or marine foodstuff, regarding both elevated $\delta^{13}C$ and $\delta^{15}N$ values, and further implied by the community's geographically location. However, in order to confirm or reject this hypothesis, a more thorough analysis of the faunal samples in this region (missing in this study) is essential to provide baseline values of the ecosystem at the time.

In either case, these points serve to illustrate stable isotope analysis as a valuable addition in diet reconstructions of past populations by targeting specific communities and its individuals.

Residents from different geographical regions exhibit various types of diets, based on different cultural views and food type availability. It is along these conclusions that, nowadays, it is possible to apply the stable isotope analysis method in a forensic set up. The interpretation of isotopic data from samples of the forensics' case in study aids the identification of an unknown individual through hypothesizing potential provenances and lifestyle/life circumstances (Fraser & Meier-Augenstein, 2007).

Ultimately, numerous local, state, federal investigative and scientific agencies recognize this technique's utility in occurrences where human identification is needed, based on the history encoded in the isotopic ratios of the unidentified decedent's tissue (Chesson et al., 2017). Although, they rely not only on the analysis of nitrogen and carbon isotopes, but also on isotopes like sulfur

(^{34}S), that equally reflect the type of food ingested, and oxygen (^{18}O), where it's possible to narrow down the food consumer's location (Bol et al., 2007).

5.1. Limitations of this study

This study confirms the saying "You are what you eat", since the isotopic values obtained from the bone collagen are a reflection of dietary intake. However, it is known that additional factors might influence the isotopic results if the consumer was under certain conditions, which can lead to erroneous interpretation (Fuller et al., 2005).

In particular, $\delta^{15}N$ values might be affected by deviations in nitrogen homeostasis due to a range of facts, from nutritional stress to pathological conditions (Alaica et al., 2019; Fuller et al., 2005). Findings from a lot of studies (Fuller et al., 2005; Hobson et al., 1993; Katzenberg & Lovell, 1999) show that in anabolic states, the nitrogen's isotopic ratio of the body protein pool tends to decrease and in catabolic states it tends to increase.

Under certain conditions like fasting and nutritional stress, the consumer's tissue becomes the main nitrogen source. During these situations, skeletal muscle protein breakdown occurs to obtain nitrogenous compounds for protein synthesis (catabolism) (Reitsema, 2013). This process results in the remaining tissues being enriched with heavy nitrogen isotopes (^{15}N) (Fuller et al., 2005; Hobson et al., 1993; Reitsema, 2013). As discussed during this thesis, nuns usually follow the Benedict's rule of fasting, so these findings caution us on the assumption of an average diet.

Regardless, if we consider that the tissue's turnover rate influences the extent of $\delta^{15}N$ enrichment in them, it can be assured that bone collagen is the tissue that most appropriately reflects diet, on the account of its slow isotopic turnover rate (Hobson et al., 1993; Katzenberg & Lovell, 1999). Yet, according to Katzenberg & Lovell (1999), it is important to avoid obvious pathological bones, since the bone has the capacity to register injuries (resulted from disease or nutritional stress), expressing it through various stable isotope ratios that consequently causes implications during diet reconstruction (Katzenberg & Lovell, 1999; Olsen et al., 2014).

5.2. Limitation of this type of technique in forensics

Nowadays in some regions, there is a remarkable amount of food that does not come from local producers as a result of international trading (Rauch et al., 2007). Even with information on dietary intake, it may not always be possible to narrow down to a precise geo-location, once there might be more regions in the world with the same isotopic profile (Fraser & Meier-Augenstein, 2007).

None the less, due to a number of worldwide studies, it is possible to identify strong isotopic-patterns concerning agriculture and diet, within and between world continents (Cerling et al., 2016; Rauch et al., 2007). So, the assumptions must rely on what reference data is known or presumed to be known at the time of the research (Fraser & Meier-Augenstein, 2007).

Interestingly, baseline dataset generated 40 years ago by Gaffney (1978) of $\delta^{13}C$ values, can still be observed in the modern days' isotopic studies (Chesson et al., 2017; Hülsemann et al., 2015; Valenzuela et al., 2012). It quantified nutritional differences between US Americans and Europeans, where individuals from New York had a diet approximately 75% based on C_4-plants food derivates, contrary to the European populations, that presented a 70% C_3-plant based diet (Chesson et al., 2017),

Lastly, this concludes that the present dataset of carbon and nitrogen stable isotopes values is progressively increasing and works as an asset for the interpretation of modern and ancient human's diet reconstruction, alongside their geographical provenance (Hülsemann et al., 2015).

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