

Extraction of soluble collagen and its feasibility in the palaeodietary research

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In current palaeodietary research, gelatinization is the main method to extract insoluble collagen (ISC) from ancient bones. However, the degradation products of ISC, i.e., soluble collagen (SC), is often neglected and abandoned. In this work, we try to separate the extracts of ancient bones using gel chromatography and compare the contents of carbon and nitrogen, atomic C/N ratio, and stable carbon and nitrogen isotopic values of the extracts from three peaks to determine which peak can be attributed to SC. At last, the potential application of SC in palaeodietary research is discussed based on the comparison of stable isotopic values between ISC and SC. Among the three peaks, the second with the retention time between 17.5 min and 27.5 min had the most broad peak shape, indicating that the molecular weights of proteins collected were most variable. Besides, the contents of carbon and nitrogen and atomic C/N ratio of extracts in this peak were closest to the corresponding ISC. Based on the above, we conclude that the extract in second peak is SC. More important, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of ISC and SC are very similar. For ISC and SC with atomic C/N ratios within the normal range (2.9–3.6), the mean difference of $\delta^{13}\text{C}$ value was only $(0.3\pm 0.2)\text{‰}$ ($n=2$) while $\delta^{15}\text{N}$ value was $(0.6\pm 0.1)\text{‰}$ ($n=2$). Although the atomic C/N ratios of some SC are slightly beyond the normal range, the mean differences of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were still only $(0.4\pm 0.1)\text{‰}$ and $(0.3\pm 0)\text{‰}$ ($n=2$) respectively. These isotopic differences are quite below the isotope fractionation in one trophic level ($\delta^{13}\text{C}$ values of 1‰ – 1.5‰ and $\delta^{15}\text{N}$ values of 3‰ – 5‰), suggesting that SC had great application potentials in palaeodietary research.

soluble collagen, insoluble collagen, gel chromatography, stable isotopic analysis

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Collagen in human or animal bones from archaeological sites contains rich potential information about age, species, diet, and living environment of individuals (Cai et al., 1984; Hu et al., 2000, 2008; Zhang et al., 2003). By stable carbon and nitrogen isotopic analysis of collagen, we can reveal human and animal diet and understand their food resources and living environment (Guan et al., 2007; Hu et al., 2009;

Zhang et al., 2010; Guo et al., 2001; Hou et al., 2012). As the collagen is the main object for stable isotopic analysis, the extraction of collagen from ancient bones has always been great concerns for scholars (Hedges, 2002; Trueman et al., 2002; Collins et al., 2002; Jørkov et al., 2007; Schweitzer et al., 2008).

Collagen in human and animal bones is composed of more than 90% organic matter. Its molecular weight is about 300 kD. Its chemical structure is quite stable and hardly

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soluble in water or diluted acid solution at room temperature. However, during the long burial period, factors such as humidity, temperature, and microorganisms would have had a great effect on bone preservation so that collagen degraded gradually or even disappeared completely (Hu, 2002). Components of insoluble collagen with large molecular weights and long peptides would be gradually reduced while components with smaller molecular weight and shorter peptides would increase and become more soluble. This process transforms more insoluble collagen (ISC) into soluble collagen (SC). In current research of bioarchaeology, gelatinization is the main method to extract ISC from ancient bones (Jay et al., 2006). The procedure is described as follows. First, the bone is fully decalcified by acid. Then gelatinization is carried out to make ISC into acid soluble gelatin based on the fact that collagen can be dissolved in diluted acid at high temperature. Finally, ISC is obtained by lyophilization. Apparently, the advantage of gelatinization aims to extract ISC with a relatively stable quality. According to the protocol mentioned above, however, only small quantity of ISC can be extracted and even no ISC can be obtained due to the poor bone preservation (Hu et al., 2010). The main reason for this is due to the great loss of ISC in poorly-preserved bones and transformation into SC that is usually discarded during the process of the gelatinization. Thus, extracting collagen using the gelatinization method greatly limits the selection of bone samples for stable isotopic analysis, which would seriously hinder our understanding of the potential information that collagen may provide.

As mentioned above, SC dissolved in acid solution is usually discarded during the process of gelatin extraction. To date little research has been done to explore the chemical differences between the gelatinized collagen and those degraded in the waste solution and isotopic variation between their carbon and nitrogen stable isotopic signatures. In addition, can SC be used for palaeodiet research as well? To answer these questions, in the present work, we attempt to: (1) separate and extract acid-soluble materials from ancient animal bones unearthed in archaeological sites using gel chromatography technology; (2) measure the contents of carbon and nitrogen and stable carbon and nitrogen isotopic values of the extracts in every tube to identify which might be SC, and (3) compare the difference of chemical properties between SC and ISC. Finally, we will discuss the potential applications of SC in palaeodietary research.

1 Materials and methods

1.1 Materials

Animal bones analyzed in the present work came from the Xiaoshuangqiao site (XSQS), which is located in Zhengzhou City, Henan Province (Henan Provincial Institute of Cultural Relics and Archaeology et al., 1996; Henan Provincial Institute of Cultural Relics and Archaeology, 2012),

which include two pigs, one sheep, and one dog. All the samples are dated to the Middle Shang period (ca. 3400 a BP). Details about location, species, and parts are shown in Table 1.

1.2 Equipment

ISC extraction and SC separation were conducted at the Key Laboratory of Vertebrate Evolution and Human Origins, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences. The chromatography system includes the followings: QuikSep-50D chromatography system (H & E), QuikSep UV-100D UV detector (H & E), QuikSep chromatography workstation (H & E). Vantage-L type chromatography column (Millipore, 500 mm×16 mm), packed with Cellufine GCL-2000 gel (Chisso, 360 mm×16 mm). The separation was under room temperature.

The contents of C and N and their isotopes ratios of extracts were measured by Isoprime 100 IRMS coupled with Elementary Vario at the Environmental Stable Isotope Laboratory, Institute of Agricultural Environment and Sustainable Development, Chinese Academy of Agricultural Sciences.

1.3 Insoluble collagen extraction

Collagen was extracted from animal bone samples according to the protocol outlined in Jay (2006) and modified somewhat in the present study. About 2 g of bone samples were collected and subject to mechanical cleaning to remove surface contaminants. The bone samples were then demineralized in 0.5 mol/L HCl at 4°C. The solution was updated every 2 days until the bone samples became soft and showed no sign of bubbles. The obtained residues were washed by deionized water until neutrality, and then rinsed in 0.125 mol/L NaOH for 20 h at 4°C, and washed by deionized water again. After that, the remains were rinsed in 0.001 mol/L HCl to gelatinize at 70°C for 48 h and filtered while it was still hot. The residues were freeze-dried for 48 h before the collagen was obtained and weighted. The collagen yield was divided by the weight of collagen to the bone weight (Table 1).

1.4 Soluble collagen extraction

SC was isolated by the method proposed by Ajle (1991) to extract protein from ancient bones and modified relatively. Two groups of pig bone samples, each equally divided into three parts, were prepared to test the replication effect of experiments. Samples were assigned as eight unique lab numbers: Y, G, Z1①, Z1②, Z1③, Z2①, Z2②, and Z2③. All samples were treated mechanically to remove surface contaminants. Approximately 1 g of bone sample was demineralized in 10 mL of 20% formic acid for 20h at 4°C and then centrifuged at 3000 rpm for 10 min. The supernatant

Table 1 Animal bones information and isotopic data from XSQS

Lab No.	Location	Species	Part	C (%)	N (%)	C/N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Collagen (%)
Y	T97④	sheep	tibia	42.4	15.1	3.3	-15.2	7.2	1.0
G	T41 M20	dog	humerus	42.8	15.1	3.3	-15.5	9.2	1.3
Z1	T137③	pig	femur	42.5	15.3	3.2	-10.8	8.2	1.5
Z2	T129 H98	pig	tibia	43.9	15.8	3.3	-10.6	8.3	1.1

was collected and preserved at 4°C.

The extracted supernatants of eight samples were divided into four groups and separated by gel chromatography with the interval of approximate 24 h. The extracted solution of the first group (Y and G) was separated immediately after centrifugation. After 24, 48, and 72 h, the supernatant solutions of the second group (Z1① and Z1②), the third group (Z1③ and Z2①), and the fourth group (Z2② and Z2③) were in turn transferred to gel for separation.

5 mL of extracted solutions were injected into the chromatography system after full balance of 20% formic acid in chromatography column with ultrapure water as eluent (flow rate at 2 mL/min). Protein peaks were detected at UV 280 nm and 5 mL eluent was collected in each tube. After lyophilization, the substance in tubes was collected and weighed (Table 2). However, there were some tubes from which no substance was collected.

1.5 Measurements of C and N, C and N stable isotope ratios

Some extracts were taken from each tube and weighed. The contents of carbon and nitrogen and their isotopic values were measured. The standard for measuring the contents of carbon and nitrogen was Sulfanilamide. IEAE-N-1 and USGS 24 were used to normalize N_2 (AIR as standard) and CO_2 (PDB as standard) in steel bottles, respectively. For every 10 samples, we interpolated a collagen sample prepared by our laboratory (average $\delta^{13}\text{C}$ value of $(14.7\pm 0.2)\%$, average $\delta^{15}\text{N}$ value of $(6.88\pm 0.2)\%$) for correction. The measurement precision for C and N was $\pm 0.2\%$. Stable isotopic data are also shown in Tables 1 and 2.

SPSS 16.0 and Origin8.0 were used for statistical analysis.

2 Results and discussions

2.1 Gel chromatograms

Figure 1 shows the gel chromatograms of different sample groups after formic acid extraction. For simplicity, only one chromatogram graph of one sample within each group was presented here. Cellufine GCL-2000 gel can easily separate the proteins with molecular weight of 3–3000 kD. In Figure 1(a), there are three peaks after Cellufine GCL-2000 gel filtration. Retention time of the 1st peak is 13–17 min with

the highest peak at about 15 min. Compared to the 1st and 3rd peaks, the 2nd peak with retention time between 17 min and 27 min was most mild. The 3rd peak is very high, with the retention time between 27 min and 50 min. However, Figure 1(b) shows clearly that the first two peaks almost entirely disappear and only the 3rd peak remains in the last two groups.

During the gel filtration, all experimental conditions are the same except the processing time. Figure 1 demonstrates that the longer the time of bone samples in acid solution is, the less the amount and types of proteins obtained are. This should be closely related to instability of proteins in acid solution. Therefore, it is important to determine the appropriate extraction time to ensure that SC was successfully extracted from the bones.

2.2 Chemical properties of the extracts

In order to explore the chemical properties of the extracts in each peak, we compare the contents of carbon and nitrogen, the atomic C/N ratio, and C and N stable isotopic values.

In Figure 2 there is a significant variation of contents of carbon and nitrogen of the extracts at different retention time, showing a pattern that the contents of carbon and nitrogen gradually increase, and then decrease sharply. In other words, the carbon contents of the extracts from the 1st peak, whose retention time is 15–17.5 min (tube 7), are approximately 25% but the nitrogen contents are generally less than 8.5%. However, the carbon contents in the 2nd peak, whose retention time is 17.5–27.5 min (tubes 8–11), increase up to about 30%–40% and the nitrogen contents increase to about 9%–14%. At last, the contents of carbon and nitrogen in the 3rd peak, whose retention time is after 27.5 min (tube 12), are dramatically reduced to less than 10% and 1%, respectively.

In addition, there is also a noticeable difference in the C/N ratio of extracts in three peaks in Figure 3. Basically, the average of C/N ratio in the 1st peak is about 4, slightly lower in the 2nd peak, and dramatically increases, even more than dozens or hundreds of times as high as that in the 3rd peak.

Moreover, some chemical differences of the peaks can also be revealed based on their large variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. In Figure 4, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of extracts in the first two peaks, whose retention times are 15–27.5 min, are relatively stable, whereas the $\delta^{13}\text{C}$ values of

Table 2 C and N contents and isotopic values of extracts from each tube^{a)}

Lab No.	Tube No.	C (%)	N (%)	C/N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Weight (mg)
Y	7	25.6	8.4	3.5	-16.1	7.3	3.7
	8	36.8	13	3.3	-15.6	8	1.5
	11	36.1	11.9	3.5	-15.8	7.6	3.8
	12	11	2.2	5.7	-17.4	6.8	10.5
	13	7.1	0.2	54.9	-21.7	4.6	208.3
	14	8.9	0.1	73.8	-21	-0.4	231.9
	15	9.7	0.1	94.3	-21.4	3.2	135.4
	16	8.7	0.4	23.1	-20.7	3.4	11.2
G	8	36.3	12.9	3.3	-15.6	9.8	3.2
	9	39.7	14.1	3.3	-15.6	9.9	3.3
	12	9.5	0.8	14.5	-20	9	27.8
	14	10.4	0.1	121.6	-21.3	8	100.3
	15	10.9	0.2	63.4	-21.2	4.4	31
Z1①	7	24.4	6.4	4.5	-12.8	8.2	5
	8	30.3	9.4	3.8	-11.6	8.3	3.8
	10	32.5	10.4	3.7	-10.7	8.6	4.8
	11	29.3	8.6	4	-11.6	8.6	4.6
	12	7.6	0.8	11.7	-17.6	7.3	32.7
	13	8.9	0.2	61.2	-21.3	3.1	198.5
	14	9.2	0.2	71.9	-20.3	4.2	181
	15	9.5	0.1	79.2	-22.1	0.5	123.5
	16	10.5	0.4	32.2	-20.8	4	5.1
Z1②	7	25.8	6.6	4.6	-12.7	8.3	4.2
	10	35.9	11.7	3.6	-10.4	8.4	4.3
	11	35.7	11.2	3.7	-10.5	8.7	4.2
	14	11.1	0.1	108.2	-21.5	8.5	261.7
	15	9.9	0.1	116	-21.7	2.3	197
	16	9.2	0.1	97.5	-21	2.7	45.1
Z1③	13	4.4	0.1	56.4	-21.6	5	115.5
	14	8.9	0.1	94.7	-22.1	5.2	204
	15	7.7	0.1	111.7	-21.2	-0.5	101.9
	16	10.1	0.1	90.6	-21.5	1.7	46.5
	17	8	0.3	37.2	-21.3	-2.3	5.3
Z2①	13	6.6	0.1	59.3	-20.8	2.2	47.3
	14	9.7	0.1	113.4	-21.2	0	118.2
	15	9.8	0.1	114.3	-21	-0.5	88.8
	16	8.3	0.1	74.3	-20.8	-2.5	29.4
Z2②	13	6.6	0.1	55	-20.9	2.7	81.1
	14	9.5	0.1	157.8	-22.2	2.8	228.1
	15	9.9	0.1	105.4	-21.4	6.4	152.1
	16	10.5	0.1	94.5	-21.6	1.1	44.1
Z2③	12	9.9	1.4	8.3	-15.5	7.8	7.9
	13	8	0.1	66.3	-20.2	3.3	113.6
	14	9.2	0.1	97.7	-21.8	-0.2	228.1
	15	10	0.1	116.9	-21.6	1.6	139.3
	16	8.4	0.2	65.6	-21.2	-2.4	19.1

a) As no any substance was produced in some tubes, the tube number without any material was not listed in the table.

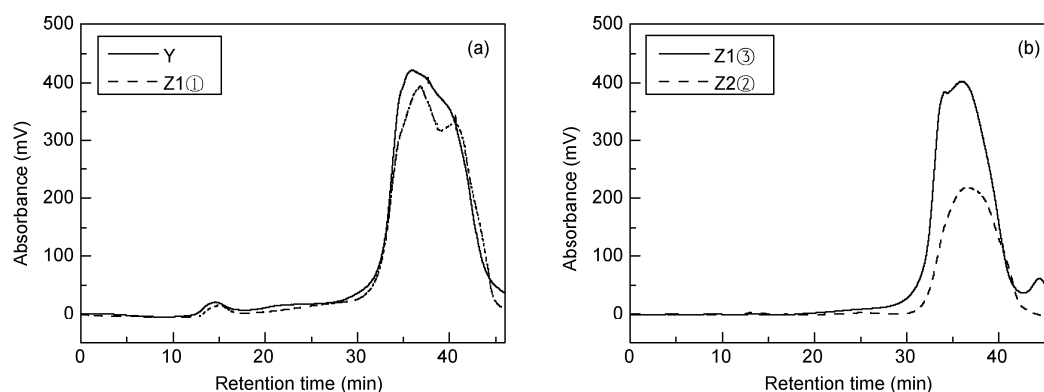


Figure 1 Cellufine GCL-2000 chromatograms.

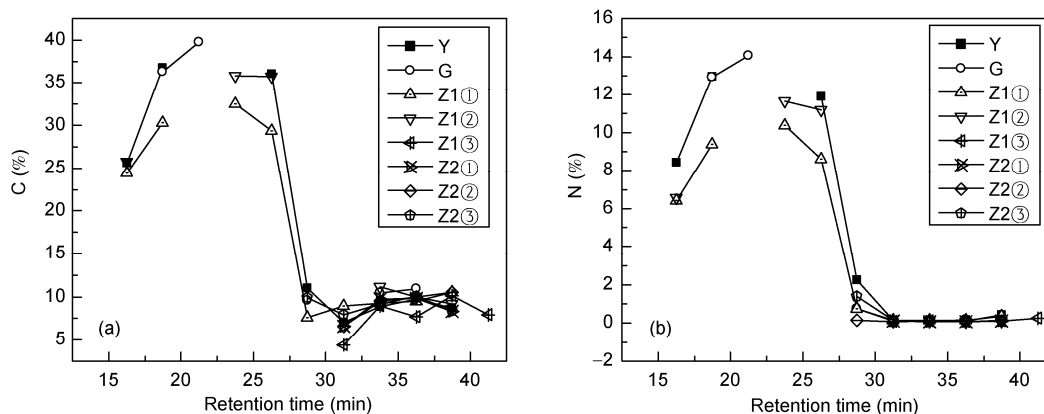


Figure 2 Measured C and N content values of extracts in each tube.

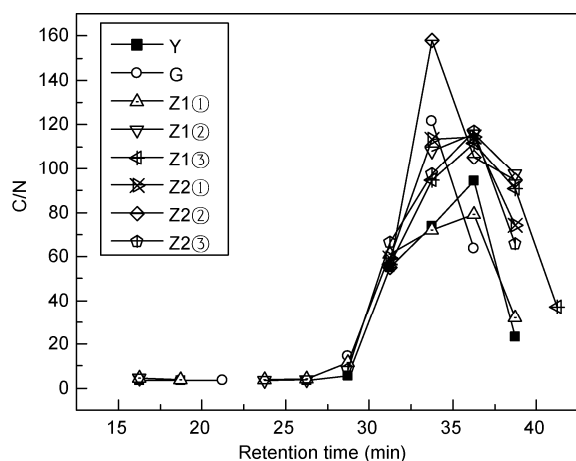


Figure 3 Measured C/N ratio of extracts in each tube.

extracts in the 3rd peak with retention time after 27.5 min are significantly low, and the $\delta^{15}\text{N}$ values show some fluctuations. Even the negative $\delta^{15}\text{N}$ values in some tubes are observed, which is obviously not in accordance with the property of animal protein. Therefore, we suppose the nitrogen contents in these tubes were too low to meet the requirements for isotopic measurements.

To sum up, not only the contents of C and N, C/N ratios but also the C, N stable isotopic values of the three peaks show significant differences, indicating that they may belong to different substances.

2.3 Identification of SC

As mentioned above, SC is the degraded products of ISC. Thus, the chemical properties of SC and ISC should be similar to each other. So we measured the contents of carbon and nitrogen, atomic C/N ratio, and stable isotopic values of extracts in three peaks, and compared them with those from ISC obtained from same bone to identify which peak can be attributed to SC (Table 3).

The carbon and nitrogen contents of modern collagen are about 41% and 15%, respectively (Ambrose, 1990), and the range of atomic C/N ratio is 2.9–3.6 (DeNiro, 1985). It is generally believed that the ISC can be considered as well-preserved if its atomic C/N ratio is located with the normal range even though there are some deviations of their carbon and nitrogen contents. Based on the above criteria all extracted ISC can be used for the stable isotopic analysis (Table 1).

In Table 3 the carbon and nitrogen contents of extracts in the 1st peak are very low and the atomic C/N ratio is

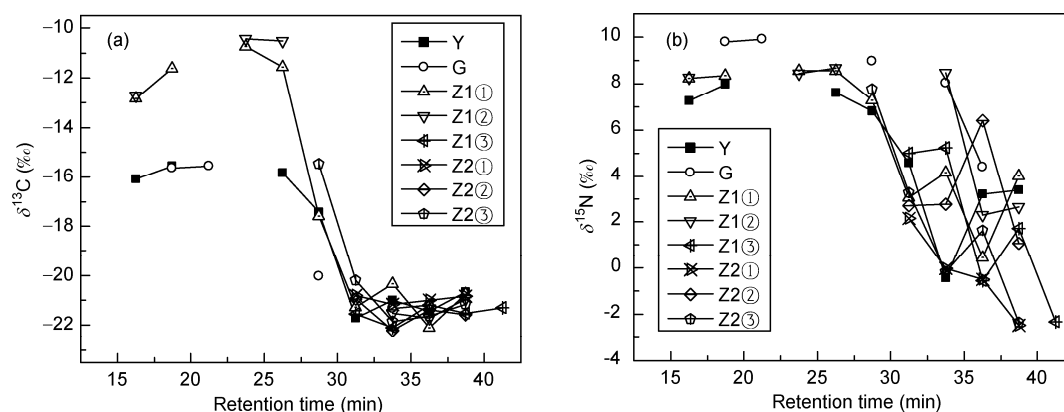


Figure 4 Measured C and N isotopic values of extracts in each tube.

Table 3 Comparison of the extracts in three peaks with ISC^{a)}

Lab No	C (%)				N (%)				C/N ratio				$\delta^{13}\text{C}$ (‰)				$\delta^{15}\text{N}$ (‰)			
	Peak				Peak				Peak				Peak				Peak			
	1st	2nd	3rd	ISC	1st	2nd	3rd	ISC	1st	2nd	3rd	ISC	1st	2nd	3rd	ISC	1st	2nd	3rd	ISC
Y	25.6	36.4	9.1	42.4	8.4	12.4	0.6	15.1	3.5	3.4	50.4	3.3	-16.1	-15.7	-20.4	-15.2	7.3	7.8	3.5	7.2
G		38.0	10.3	42.8		13.5	0.4	15.1		3.3	66.5	3.3		-15.6	-20.9	-15.5		9.8	7.1	9.2
Z1①	24.4	30.7	9.2	42.5	6.4	9.4	0.3	15.3	4.5	3.8	51.2	3.2	-12.8	-11.3	-20.4	-10.8	8.2	8.5	3.8	8.2
Z1②	25.8	35.8	10.1	42.5	6.6	11.4	0.1	15.3	4.6	3.7	107.2	3.2	-12.7	-10.5	-21.4	-10.8	8.3	8.5	4.5	8.2
Z1③			7.8	42.5			0.1	15.3			78.1	3.2			-21.5	-10.8			1.8	8.2
Z2①			8.6	43.9			0.1	15.8			90.3	3.3			-20.9	-10.6			-0.2	8.3
Z2②			9.1	43.9			0.1	15.8			103.2	3.3			-21.5	-10.6			3.2	8.3
Z2③			9.1	43.9			0.4	15.8			70.9	3.3			-20.1	-10.6			2.0	8.3

a) The extracts in some peaks of G, Z1③, Z2①, Z2②, and Z2③ are too low to measure and are not listed in the table.

significantly higher than 3.6 except Y, compared to those of ISC obtained from the same bone. On the other hand, the mean differences of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between extracts and ISC are 1.6‰ and 0.1‰ ($n=4$), respectively. It can be suggested that the chemical property of extracts in the 1st peak is similar to ISC to some degree, but not the same. The 1st peak has the shortest retention time, which indicates the molecular weight is the largest. Given the fact that low contents of carbon and nitrogen and high atomic C/N ratios, we suggest that the substances in 1st peak might mainly contain the proteins which have high molecular weights and are easily soluble in acids, probably resulting from the mixture of proteins from soils and a small amount of SC.

Among all extracts, those in the 2nd peak have the highest contents of carbon and nitrogen, and are closest to ISC obtained from the same bone. The atomic C/N ratios of the 1st group (Y and G) fall within the normal range (2.9–3.6), whereas those of the 2nd group (Z1① and Z1②) are slightly higher. The mean difference of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between SC and ISC is only (0.3 ± 0.2) ‰ and (0.5 ± 0.2) ‰ ($n=4$). Therefore, the extracts in 2nd peak are the

most similar to ISC. On the other hand, the peak shape is quite flat, suggesting that the protein molecular weights are very diverse, indicating that they are composed of peptide chains with variable lengths, which is in accordance with the characteristics of SC. So we infer that the extracts in 2nd peak are SC. Moreover, we also analyzed the amino acids composition of extracts in this peak in other paper, which is fully consistent with the characteristic of collagen.

The C and N contents of extracts in the 3rd peak are significantly lower than those in ISC, and the atomic C/N ratios are much higher too. Meanwhile, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of extracts are very different from those in ISC. These lines of evidence imply that the extracts were not SC. Furthermore, the 3rd peak had the longest retention time, indicating that the molecular weights of extracts in this peak are the smallest in all extracts of three peaks. The strongest absorption at UV 280 nm might be caused by the strong interferences by purine, pyrimidine, and nucleic acids in the extract (Wang et al., 2002). Accordingly, we suggest that the extracts in 3rd peak should be the mixtures with low molecular weights, including purine, pyrimidine or nucleic acids.

2.4 Comparison of SC and ISC extraction rate

In the long-term burial, factors such as humidity, temperature, and microorganisms would have a great impact on bone preservation. The collagen degraded gradually, resulting in that the extraction rate of ISC by gelatinization method is often low, much lower than that collagen from fresh bones (20%) (Hu et al., 2005, 2010). The comparison of extraction rate between SC and ISC is plotted in Figure 5.

It is easily seen that in Figure 5 the extraction rates of SC are higher than those from ISC. Specifically, the extraction rates of ISC are between 1.0% and 1.5% with the mean value of 1.2%, whereas those from SC are between 1.9% and 3.6% with the mean values of 2.6%. Obviously, bone collagen has been largely degraded during the long-term burial and transformed from ISC into SC. So, it is very important and meaningful to explore the potential application of SC in palaeodietary research. Furthermore, the reason why the extraction rate of Z1 varied between the first and second extractions may be caused by the fact that SC was more degraded due to the longer immersion in acids.

2.5 Comparative analysis of SC and ISC in C and N contents and stable isotope ratios

The differences of the contents of C and N and their stable isotopic values between SC and ISC are the key to determining whether SC can be applied in the palaeodietary research or not.

Figure 6 is the line chart of the contents of C and N, the atomic C/N ratios of SC and ISC. In Figure 6(a) the contents of C and N of ISC are higher than SC, showing that the chemical properties of SC are not stable enough. Figure 6(b) presents that the atomic C/N ratios of SC extracted from the 1st group (Y and G) are similar to ISC and located within the accepted range for uncontaminated collagen (2.9–3.6). However, with the extraction time prolonged, the atomic C/N ratios of SC obtained from the 2nd group (Z1① and Z1②) significantly increase and even exceed the range of uncontaminated collagen. As showed above, the longer

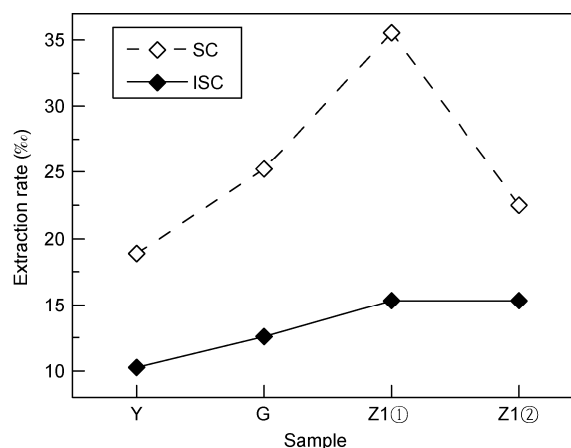


Figure 5 Comparison of SC and ISC in the extraction rate.

the immersion time in the formic acid for the bones is, the more easily the SC is degraded. Especially the N in SC is more washed away than C.

Figure 7 is the line chart of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of SC and ISC. Figure 7(a) shows that the $\delta^{13}\text{C}$ values of two collagens from the 1st group (Y and G) are very similar. Its mean difference between SC and ISC is only $(0.3 \pm 0.2)\text{‰}$ ($n=2$). Although the atomic C/N ratios of SC obtained from the 2nd group (Z1① and Z1②) are slightly beyond the normal range, the mean difference of $\delta^{13}\text{C}$ values is only $(0.4 \pm 0.1)\text{‰}$ ($n=2$), still similar to ISC. Figure 7(b) presents that the $\delta^{15}\text{N}$ values of SC are slightly higher than ISC. Specifically, the average difference of $\delta^{15}\text{N}$ values between two collagens of the 1st group (Y and G) is about $(0.6 \pm 0.1)\text{‰}$ ($n=2$), and $(0.3 \pm 0)\text{‰}$ ($n=2$) in the 2nd group.

Our study shows that the differences of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between SC and ISC obtained from the same bones were only slightly higher than the m accuracy ($\pm 0.2\text{‰}$) of the instrument and far below the isotopic fractionation in one trophic level ($\delta^{13}\text{C}$ value of 1‰ – 1.5‰ and $\delta^{15}\text{N}$ values of 3‰ – 5‰) (DeNiro, 1985; Ambrose, 1990). This suggests that SC can be also reliably used in the palaeodietary research in principle.

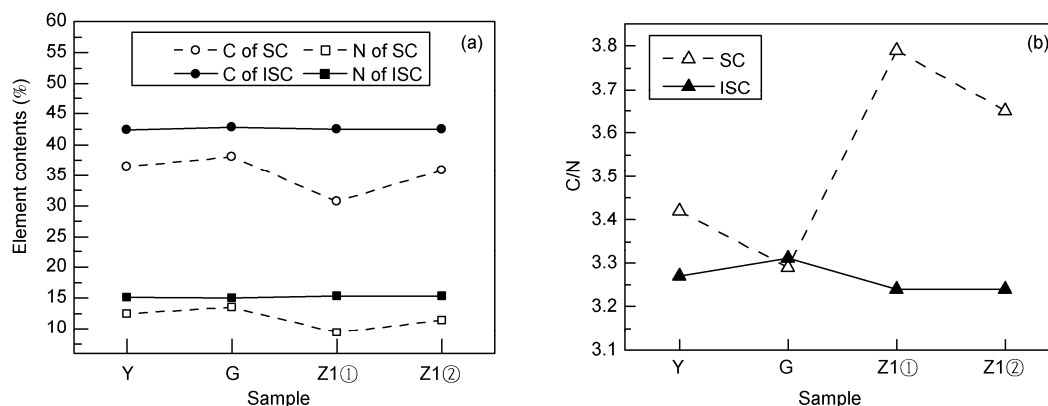


Figure 6 Comparison of SC and ISC in the contents of C and N, the atomic C/N ratio. (a) C and N contents; (b) atomic C/N ratio.

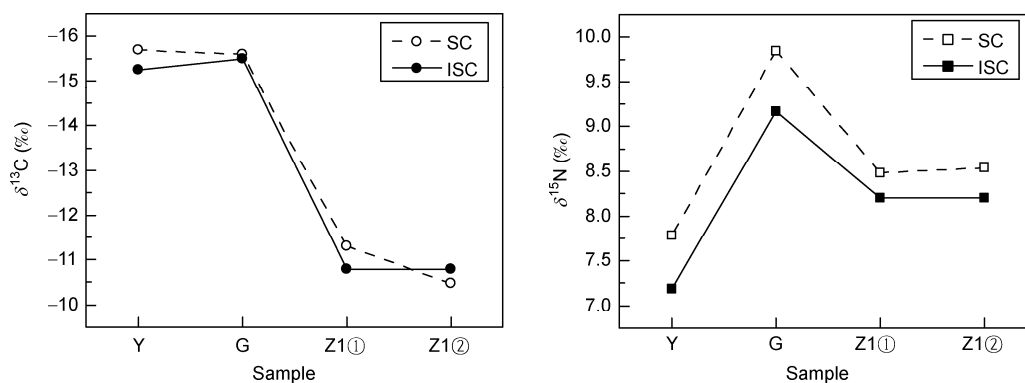


Figure 7 Comparison of SC and ISC in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

To interpret the small differences in the C, N stable isotopic values between two collagens, we propose the following explanations: 1) SC was the degradation products of ISC, which consisted of diverse peptides. The composition of amino acids and the C, N contents of two collagens are probably different (Hare et al., 1991; O'Connell et al., 2001; Buckley et al., 2008); 2) there may be also mixed with other proteins with similar molecular weights in SC. In order to explore the differences further, we have carried out amino acids analysis of two proteins and the results will be discussed later.

2.6 The prospect of soluble collagen in the study of palaeodiet

Most collagen in human and animal bones usually was degraded and decomposed during long-term burial, which makes it difficult to extract enough amount of uncontaminated collagen by gelatinized method (Hu et al., 2010). This problem seriously limits sampling strategy in the palaeodietary research and fails to reveal the potential information in ancient bones.

The present study confirms that the contents of C and N and atomic C/N ratio of SC in ancient bones are all satisfied with the criteria of uncontaminated protein. More important, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of SC and ISC are very close, implying that C and N stable isotopic analysis of SC can also be used in the palaeodietary research when ISC is not available in bones under poor preservation condition. Obviously, it has extremely important theoretical and practical significances to continue the research of SC, which will allow us to expand the bone sampling and promote the development of the palaeodietary research.

3 Conclusions

In this paper, two methods, gel chromatography and gelatinization, were used to obtain SC and ISC from ancient bones. Based on the comparison of the contents of C and N, C and

N stable isotopic values of two proteins, the following conclusions can be drawn.

(1) The extracts could be divided into three peaks by gel chromatography, among which the 2nd peak was SC.

(2) The contents of C and N of SC are slightly lower than ISC whereas the atomic C/N ratios are slightly higher.

(3) The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of SC and ISC are very similar, which suggests that SC also could be applied to the palaeodietary research.

Although SC has a great potential in the palaeodietary research, it should be noticed that the chemical properties of SC are not as stable as ISC. This work shows that the contents of SC could be significantly reduced and N was washed away more if the acid processing time was too long. Thus, the future research will focus on determining the appropriate extraction conditions like acid concentration, extraction time, and gel separation. In addition, there are still more chemical differences between SC and ISC needed for further study, such as isotopes of single amino acids, H and O isotopes.

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