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# Diet communication on the early Silk Road in ancient China: multi-analytical analysis of food remains from the Changle Cemetery

Meng Ren<sup>1</sup>, Renfang Wang<sup>2</sup> and Yimin Yang<sup>3\*</sup> 

## Abstract

Characterization of ancient food remains could reflect how people exploited biological resources and interacted with different cultures in antiquity. In this study, Fourier Transform Infrared Spectroscopy (FTIR), starch grain, phytolith, stable isotope analysis and proteomics were adopted to characterize the composition of the food remains including three cereal foodstuffs, some meat strips and a kebab discovered at the Changle Cemetery (202 BCE–220 CE), Ningxia, northwest China, a key area on the Silk Road. The results show that the cereal foods were baked cakes, primarily made from foxtail millet (*Setaria italica*). One cake was simply made of millet grains, the others were more elaborately comprised of millet flour with the addition of barley flour and meat from cattle (*Bos taurus*) and chicken (*Gallus gallus*). These findings indicate that grinding and baking technologies, which were considered as the culinary tradition in western Eurasia, had been adopted at the latest by the inhabitants in Ningxia region at that time. The large-scale migration from central China to Ningxia and the opening up of the Silk Road during the Han Dynasty gave rise to the diet communication and assimilation in this border area. Moreover, the meat strips were identified as dried beef, and the kebab was likely to be made from sheep/goat meat, wherein the latter is the earliest scientific evidence of mutton kebab in ancient China up to our knowledge. The analytical strategy could be widely applied in characterizing ancient visible food remains in order to better understand their preparation methods.

**Keywords:** Ancient foodstuff, Baked cake, Kebab, Culture communication, Proteomics

## Introduction

Food production is fundamental to human society, and archaeological foodstuffs can provide straightforward information regarding ancient diets and food culture. Due to distinctive geographical environments, economic patterns and cultural traditions, different societal groups tend to have different characteristics concerning food processing activities. It is widely accepted that grinding-and-baking technologies were the primary cooking methods in western Eurasia (the Near East and the Mediterranean), while boiling-and-steaming were

the main methods in eastern Eurasia (China and the Far East), with overlapping cooking patterns in South Asia [1]. Several studies have been conducted to investigate the composition and manufacture of ancient crop foods found in northwest China, which connects central China and western Eurasia. Some ancient cakes excavated from the Subeixi Cemetery (500–300 BCE) were identified to be made from common millet (*Panicum miliaceum*) by baking [2]. The desiccated foodstuffs in the Yanghai Cemetery (approximately 600 BCE) were cooked dough food made from wheat (*Triticum aestivum*) and barley (*Hordeum spp.*) [3]. In addition to plant microfossil methods (starch grain and phytolith analysis), proteomics was also adopted to investigate the cereal food from the Subeixi site and showed that it was a sourdough bread made from barley and broomcorn millet by leavening with baker's

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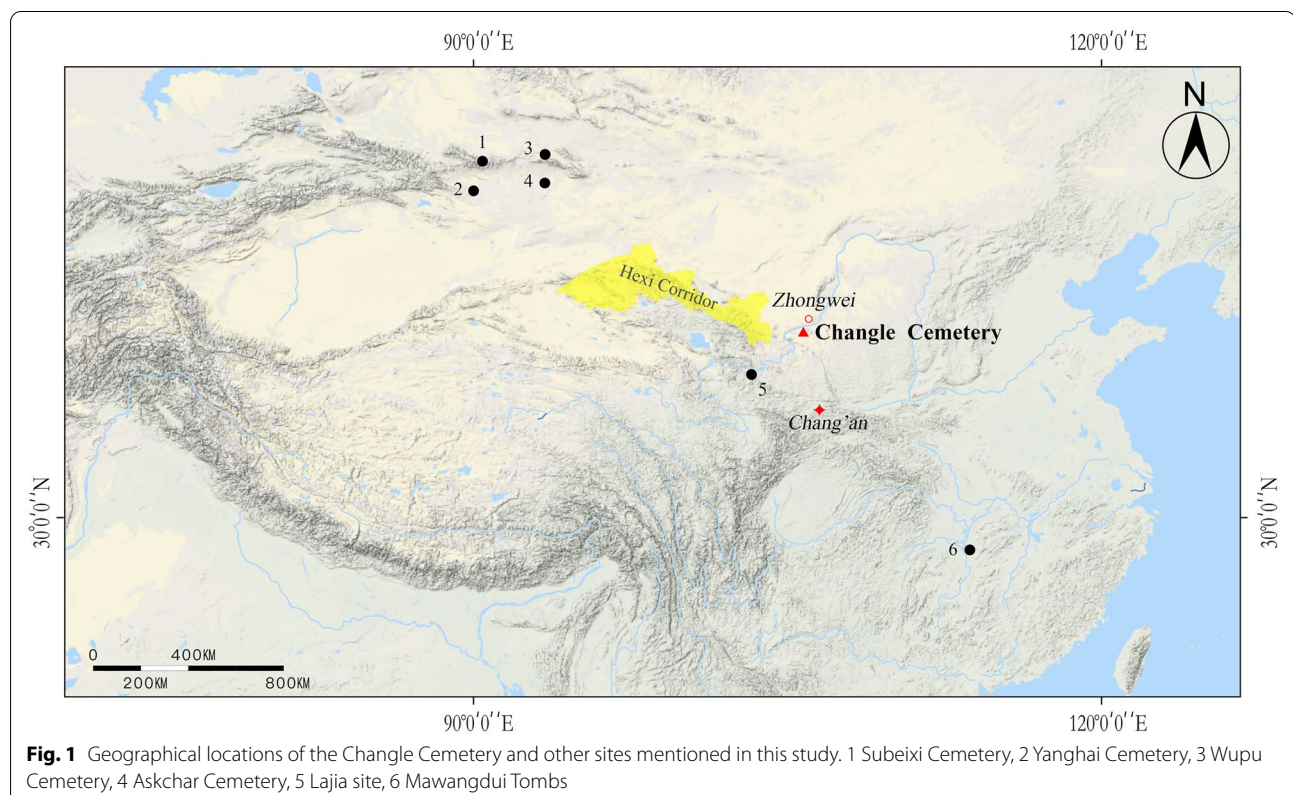
yeast and lactic acid bacteria [4]. Moreover, earlier food remains resembling baked cakes were unearthed from the Wupu Cemetery (3000–2400 cal BP) [5, 6] and the Askchar Cemetery (about 3000 BP) [7] in Hami Region in northwest China, but they have not been scientifically analyzed up to date. These studies suggest that grinding and baking techniques were widespread among the nomadic people in Xinjiang area, northwest China, at least 2500 years ago. Also, cereal flour-based food has a long history in China, such as the 4000-year-old noodles discovered from the Lajia site, which were proved to be made from millet flour [8].

Meat was also an important source of food, and the principal domestic animals in ancient China included horses, cattle, sheep/goats, pigs, dogs and chickens [9]. The evidence of consuming animal products has been primarily based on stable isotope analysis of human remains and zooarchaeological research, owing to the poor preservation conditions of ancient animals' soft tissues and their products, as well as the restriction of analytical techniques. Recently, stable isotope analysis [10], lipid analysis [11] and proteomics [4, 12] have been utilized and improved for the identification of organic materials in food remains.

According to *Hou Han Shu* (后汉书), baked food such as *Hu bing* (胡饼) had been introduced into central

China and became popular among the Han people at least in the Eastern Han Dynasty (25–220 CE) [13]. Recent research on visible food remains, however, has been restricted in the western part of China, especially in the Turpan region, where organic remains are well preserved because of the arid climate. More work is needed in adjacent regions to better understand the introduction of grinding and baking techniques to the eastern region of ancient China.

The Ningxia Hui Autonomous Region is located in the mid-northern part of China in the upper Yellow River valley. The northern minorities have been concentrated in this region since ancient times, and during the Western Han Dynasty (202 BCE–8 CE), this region became the key area of immigration from central China. With the prosperity of the Silk Road, Ningxia region acted as an important communication centre between the eastern and western parts of ancient China, which led to the diversity of diet cultures in the indigenous population, and these diet cultures inevitably influenced one another in this region. Some processed food remains, including possible cereal foods, meat strips and kebabs, were unearthed at the Changle Cemetery (Figs. 1 and 2) in Ningxia, which could provide direct evidence for the dietary communication among different populations in







the surrounding areas, and its influence on the diet culture in central China.

Human diet has become complicated with the development of society, and ancient foods were accordingly more elaborate with a greater diversity of ingredients. Therefore, a multi-analytical procedure was adopted to perform a more comprehensive examination on the archaeological food remains excavated at the Changle Cemetery. In this study, micro-samples were firstly submitted to FTIR analysis to characterize their major constituents, which were used to guide further analyses. The cereal foodstuffs were then analyzed by plant microfossil observation to determine their composition and processing technologies. Stable isotope analysis and proteomics

were performed to obtain more detailed component information.

## Materials and methods

### Materials

The Changle Cemetery (37°26′24.8″ N, 105°05′43.0″ E) is located 2.5 km south to the Changle Town in Zhongwei City, Ningxia region (Fig. 1). From May to November 2012, the field team conducted the fourth archaeological excavation of this site, and 24 tombs were discovered. 15 tombs are medium cave-type graves, whereas the others are small rectangular earthen pit graves. The Changle Cemetery dates back to the Han Dynasty (202 BCE–220 CE), when Zhongwei was under the jurisdiction of the

Anqing Prefecture in the border area. Most of the funeral objects are bronze coins and potteries, and the identified animal bones include pigs, sheep/goats, cattle, a dog, chickens, and other poultry bones. In terms of the features of the tombs and funeral objects, the owners were possibly immigrants from central China [14].

Some food remains were found in Tomb M17, which is a cave-type grave with sloping tomb-passage. The coffin chamber was surrounded by wooden pillars with thick planks covered, and the funeral objects contained potteries, *wuzhu* (五铢) coins, woodenware, lacquerware, a wooden house model, a wooden staff statue, and plant seeds. The human body was completely wrapped in a shroud, so physical anthropology examination has not been carried out until now. It is worth mentioning that the wooden staff statue was the status symbol of the elder, indicating that the owner was a venerable old man.

Five food samples were investigated in this study, including three possible cereal food remains and two meat remains (labelled as CL 1–5, Fig. 2; Table 1). The cereal foodstuffs were found in two lacquerwares placed on the ground in front of the coffin. Specifically, CL 1 was placed in one lacquer bowl, CL 2 and CL 3 were in another one. The meat remains were found in a bamboo basket on the coffin lid. In addition, modern foxtail millet (*Setaria italica*) was also selected for comparison studies.

#### Optical observation

The cross-sections of the archaeological food samples were observed under a VHX-600 digital microscope (Keyence, Japan) and photographed at 50× magnification.

#### FTIR

Infrared spectra were recorded using a Fourier Transform Infrared Spectrometer (Nicolet 6700, USA). Samples were analyzed as KBr pellets in transmission mode. Spectra were acquired over the range of 4000–400 cm<sup>-1</sup>, with a resolution of 4 cm<sup>-1</sup>, 32 scans per spectrum. The software OMNIC 8.0 was applied to deal with data.

#### Plant microfossil analysis

(1) Starch grain analysis. A little material was scraped by a clean lancet into a 1.5 mL Eppendorf tube and soaked

in 1 mL deionized water for several hours. After shaking, the slide was made using 1:1 glycerin/water. Then slides were viewed and photographed in both transmitted light and polarized light under 500× magnifications. (2) Phytolith analysis. Each sample was placed in a beaker with 5 mL nitric acid, and stirred occasionally until the reaction ceased. Then the beaker was heated at 70°C for 3 h, and a small amount of nitric acid was added several times during the period. The solutions were centrifuged at 3000g for 5 min, decanted and rinsed twice with deionized water. After drying at room temperature, the phytolith sediments were mounted onto microscopic slides in Canada balsam for photomicrography. Slides were viewed under 500× magnifications.

#### Cooking experiments

In order to figure out the cooking technique of ancient cake samples, modern foxtail millet was subjected to three processing methods: boiling, baking, and steaming. (1) The cereal was ground, mixed with water and kneaded into noodles, then put in boiling water for about 5 min. (2) The ground cereal was kneaded to dough and steamed for 10 min. (3) The ground cereal was kneaded to dough and baked at 200°C for 20 min. After being cooked, each sample was treated in the same way as the archaeological samples for starch grain analysis.

#### Stable carbon and nitrogen isotope analysis

Each sample portion was placed in a beaker with 0.1 M hydrochloric acid to remove carbonates. Then the sample was washed to neutrality, freeze-dried, and ground into fine powder. Stable isotope analysis was performed by an IsoPrime 100 IRMS (Elementar, UK) mass spectrometer coupled with an Elementar Vario (Elementar, UK). Isotope ratios (<sup>13</sup>C/<sup>12</sup>C or <sup>15</sup>N/<sup>14</sup>N) are expressed as δ in per mil (‰) relative to the internationally defined standards for carbon (Vienna Pee Dee Belemnite, VPDB) and nitrogen (Ambient Inhalable Reservoir, AIR). The measurement errors were less than ± 0.2‰ for both δ<sup>13</sup>C and δ<sup>15</sup>N values.

**Table 1** Description of the archaeological food remains from the Changle Cemetery

Archaeological code	Sample code	Description
12SCM17-5	CL 1	Hemispherical cake mainly made from loose grains, brown in color
12SCM17-4-1	CL 2	Flat cake made from cereal flour, brown in color
12SCM17-4-2	CL 3	Irregularly shaped cake made from cereal flour, brown in color
12SCM17-14	CL 4	Kebab, a small piece of meat pierced on a wooden skewer
12SCM17-5-1	CL 5	Dried meat strips

### Proteomic analysis

For each food remain, a subsample of about 10 mg was suspended in 100  $\mu$ L of extracting solution (Tris-HCl, pH 8.0, 10 mM dithiothreitol, 10% sodium dodecyl sulfate and 0.0025% bromophenol blue). The mixture was subjected to ultrasonic baths ( $3 \times 15$  min) and incubated for 1 h at 56 °C. Then the sample was sonicated again for 15 min and centrifuged for 15 min at 12,000g.

5  $\mu$ L of glycerol was added to 45  $\mu$ L of the supernatant, heated at 95 °C for 5 min. After cooling to the room temperature, the extracting solution was loaded onto the gel (SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis) with 25  $\mu$ L each well. The electrophoresis apparatus was connected to a 200 V power. The gel with sample was immersed in the staining solution (0.25% Coomassie Blue w/v, 50% ethanol, 10% acetic acid) and incubated in the microwave oven at medium-low heat for 45 s followed by slowly shaking for 10 min. The gel was washed with water for several times, and destained in the solution (25% ethanol, 8% acetic acid) until protein bands were visible.

The gel with protein band was cut into small particles and washed by distilled water three times. Then the gel particles were destained with 50% acetonitrile/25 mM  $\text{NH}_4\text{HCO}_3$  and alkylated in the dark with 50 mM iodoacetamide for 30 min. The gel pieces were then washed with 25 mM  $\text{NH}_4\text{HCO}_3$  buffer twice and immersed in 12.5 ng/ $\mu$ L trypsin solutions. The digestion was incubated in the microwave oven at 850 W for 1 min.

The digested sample was re-dissolved in 0.1% formic acid (buffer A), and analyzed by ChromXP nano LC column (75  $\mu$ m  $\times$  15 cm, ChromXP C18-CL 3  $\mu$ m 120 Å) from Eksigent. The gradient ran at 300 nL/min from 5 to 80% buffer B (0.1% formic acid in acetonitrile) over 1 h. The parameters of Triple TOF 5600 + mass spectrometer: curtain gas: 30; GS1: 4; ion spray voltage: 2.3 kV; TOF MS spectrum acquisition time: 0.25 s ( $m/z$  350–1250); a rolling collision energy voltage was used for CID fragmentation MS/MS scans; IDA number: 30; MS/MS spectra acquisition time: 0.1 s ( $m/z$  100–1500); dynamic exclusion time: 25 s.

The MS/MS spectra were searched against the NCBI nr database (released 201,604; 87,376,087 sequences; 32,052,401,578 residues) by Mascot software version 2.4.1 (Matrix Science, UK). Trypsin was selected as the proteolytic enzyme and two missed cleavages were allowed. Carbamidomethylation (C) was selected as fixed modification. Acetyl (Protein N-term), deamidation (NQ), Gln  $\rightarrow$  pyro-Glu (N-term Q) and oxidation (M) were selected as variable modifications. A peptide tolerance of 10 ppm and a product ion tolerance of 0.05 Da were used in the searches and the peptides were filtered with significance threshold  $p < 0.05$  and ions score cut-off 25. In order to

check the species-specificity of the sequences, each peptide sequence was submitted to the protein Basic Local Alignment Search Tool (BLASTp tool) in the NCBI nr database available on the website of National Center for Biotechnology Information (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

## Results

### FTIR analysis

Samples CL 1–3 show similar infrared spectra with the presence of starch (Fig. 3A). The peak at approximately  $1154\text{ cm}^{-1}$  was assigned to C–O stretching region, the peak at  $1080\text{ cm}^{-1}$  was assigned to C–O–H bending vibration region, and the peak at  $1030\text{ cm}^{-1}$  was assigned to C–O bending region [15]. This pattern of absorption peaks was characteristic of the starch moiety and indicated that they were starch-rich foodstuffs. The peak around  $1630\text{ cm}^{-1}$  was attributed to C=C stretching frequency, which suggests that these cereal foods might have been processed by heating [16]. The bands at approximately 1650 and  $1540\text{ cm}^{-1}$  in the infrared spectra (Fig. 3B) obtained from the other two samples (CL 4 and CL 5) were characteristic to amide groups (–N(H)–C=O–) [17], indicating proteinous products.

### Phytolith analysis

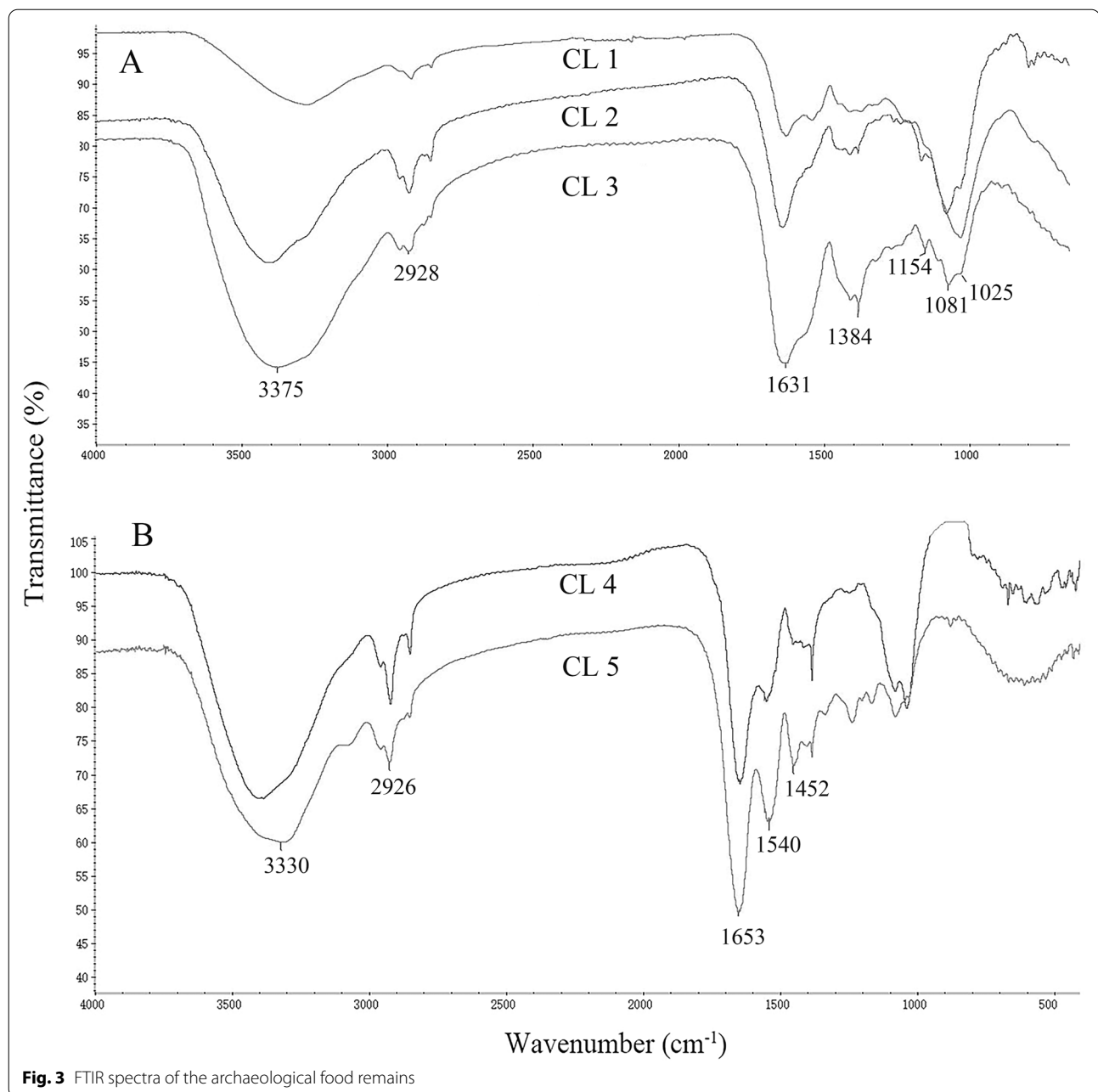
Compared with literature data [18], phytoliths in the archaeological cereal samples (Fig. 4) showed characteristics of *Setaria italica*. In detail, there are regularly arranged papillae on the surface of the sample, the epidermal long cell walls are  $\Omega$ -undulated (i.e., the undulations are rounded, wider towards the apex and narrower at the base), and the ending structure of the epidermal long cell is a “cross wavy type” (i.e., the dendriform epidermal long cell endings join others in a wavy pattern). Thus, it is deduced that the main component of three starch food remains (CL 1–3) was foxtail millet.

### Starch grain analysis and cooking experiments

Most of starch grains observed in samples CL 1–3 were damaged and lacked morphological characteristics (Fig. 5). The starch grains were mainly brownish and were pasted in clusters. They were swollen and partially gelatinized, with indistinct or an otherwise loss of cross-extinction properties under a polarized microscope. Previous studies have shown that cooking can lead to significant morphological changes in starch grains [19, 20]. Based on the results of phytolith analysis, modern foxtail millet was used as a reference to examine the cooking process of the archaeological samples.

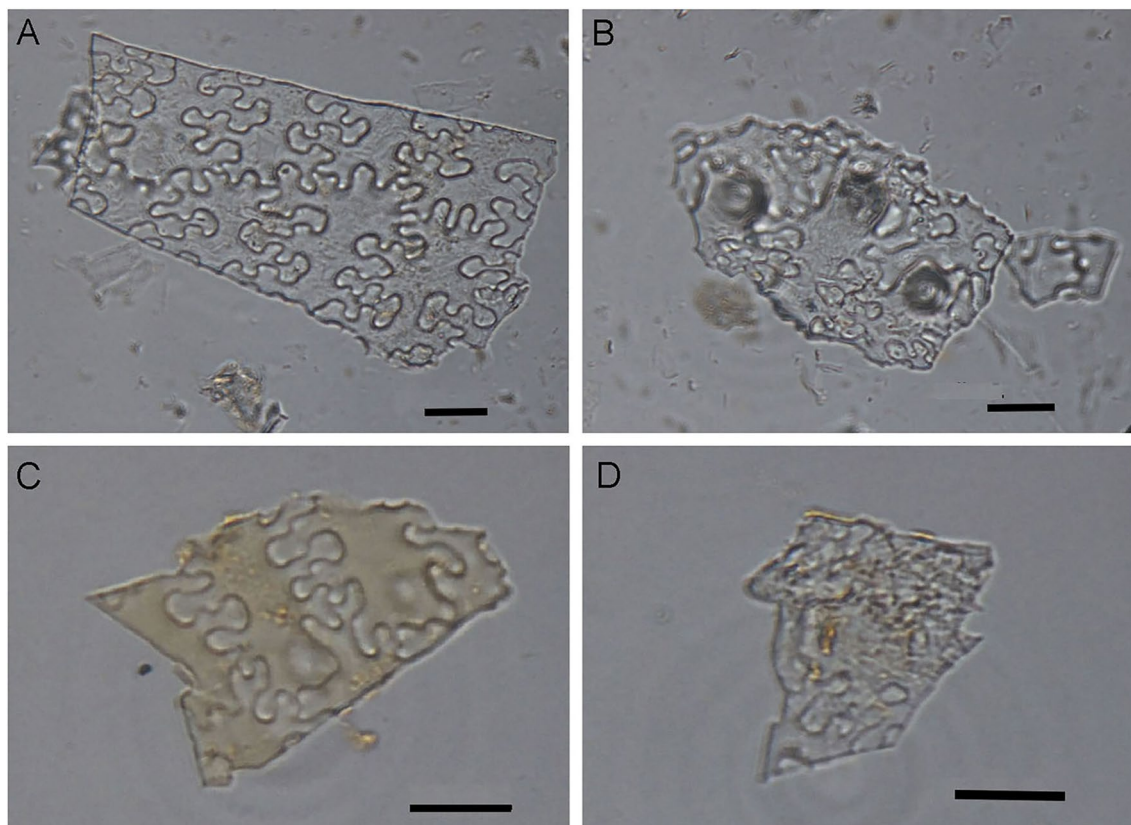
The starch grains from modern foxtail millet that were obtained through different cooking methods are shown in Fig. 5. Boiling caused swelling, collapse and





gelatinization of the starch grains, with an almost complete loss of the cross-extinction. Meanwhile, the starch grains were pasted together and had vague outlines (Fig. 5C, D). Steaming, similar to boiling, caused extensive swelling and gelatinization of the starch grains, with an almost total loss of the cross-extinction. Most of the steamed starch grains were completely damaged, and the partly-damaged grains became puckered, curved and irregularly-shaped (Fig. 5E, F). Regarding baking, the starch grains became pasted in clusters, swollen and

gelatinized, and exhibited damaged cross-extinction or sometimes a loss of extinction (Fig. 5G, H). In addition, the baked granules were brown, which was likely caused by the Millard reaction, which is a type of non-enzymatic browning between amino acids and reducing sugars [21]. This phenomenon of colour change was also found in the baking experiments of wheat flour [20], but not observed in other cooking methods. Accordingly, the appearance of the archaeological starch grains (Fig. 5I–L) exhibited a close resemblance to that of baked samples,



**Fig. 4** Phytoliths from the archaeological cereal samples: **A, B** CL 1, **C** CL 2, and **D** CL 3. Scale bars: 20  $\mu\text{m}$

which implies that three cereal remains CL 1–3 should be cooked by baking.

#### Stable carbon and nitrogen isotope analysis

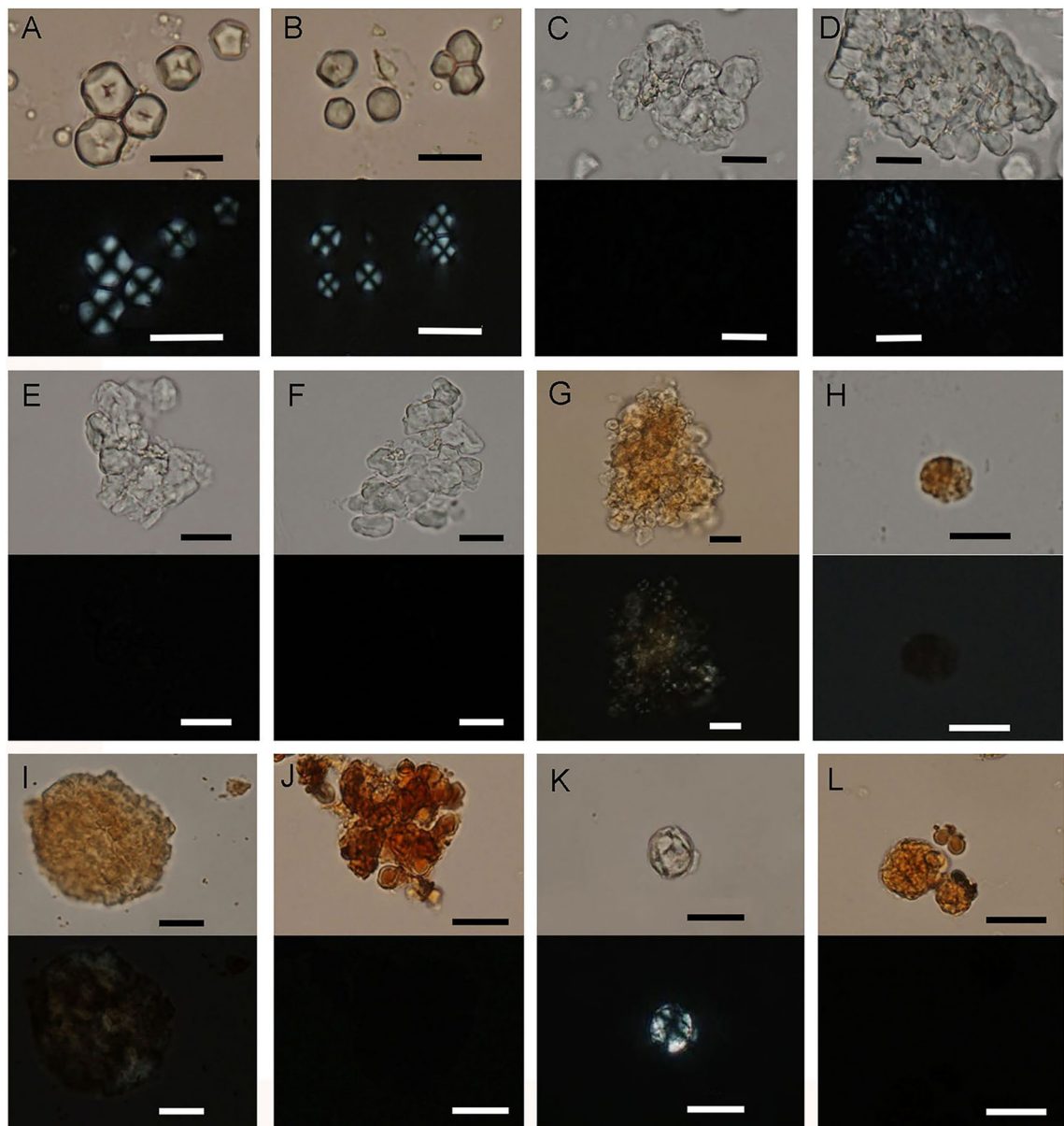
There are essentially three types of plants in the natural world according to different photosynthetic pathways, namely,  $C_3$ ,  $C_4$ , and CAM plants. The variation in  $\delta^{13}\text{C}$  among these plants is significant, with  $C_3$  plants having the lowest  $\delta^{13}\text{C}$  ( $\sim -26.5\text{‰}$ ),  $C_4$  plants having the highest  $\delta^{13}\text{C}$  ( $\sim -12.5\text{‰}$ ), and CAM plants having a mixed isotopic composition [22, 23]. Foxtail millet (*S. italica*) and common millet (*P. miliaceum*) were the major crops in ancient northern China, and both are  $C_4$  plants [24, 25]. The fractionation of  $\delta^{15}\text{N}$  is dominated by a trophic-level effect, which leads to an approximate 3–5‰ increase associated with the ascending step in the food chain [26–29].

Table 2 shows the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the food remains. The  $\delta^{13}\text{C}$  values of the cereal food samples (CL1–3) range from  $-12.09\text{‰}$  to  $-11.55\text{‰}$ , which indicates that they were predominately comprised of  $C_4$  plants and are consistent with the results of the phytolith analysis. The meat sample (CL 5) had a lower  $\delta^{13}\text{C}$  value,

which indicated some input of  $C_3$  plants in the animal's diet. The  $\delta^{15}\text{N}$  values of CL 2 and CL 3 were higher than that of CL 1, and similar to the  $\delta^{15}\text{N}$  value of the meat sample, implying the addition of animal proteins.

#### Proteomic analysis

Proteomic identification was then performed to characterize the nature and species of the protein materials in these foodstuffs. The LC/MS/MS results listed in Additional file 1: Table S1 show that collagen and actin were the major proteins identified in the meat samples (CL 4 and CL 5), besides the disregarded human background proteins. Notably, the proteins from plant and animal materials were simultaneously detected in CL 2 and CL 3, including amylase, starch synthase, myosin, actin, muscle auxiliary protein, collagen and others. Amylase is an enzyme for starch and glycogen hydrolysis;  $\beta$ -amylase is particularly present in higher plants and does not exist in mammals [30]. Collagen is the main component of connective tissue that is mostly found in tendons, ligaments and skin, and collagen type I (collagen  $\alpha 1$  type I and collagen  $\alpha 2$  type I) is the most abundant collagen in vertebrates [31]. Actin is a structural protein of microfilaments



**Fig. 5** The morphology of starch grains from modern foxtail millet (*S. italica*) and archaeological cereal samples: **A, B** uncooked modern *S. italica*, **C, D** modern boiled *S. italica*, **E, F** modern steamed *S. italica*, **G, H** modern baked *S. italica*, **I, J, K, L** CL 1, CL 2, and CL 3. The upper row of photographs showing starch grains viewed under transmitted light, and the lower row showing the corresponding grains under polarized light. Scale bars: 20  $\mu$ m

**Table 2** The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the archaeological food remains from the Changle Cemetery

Sample	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Carbon content (%)	Nitrogen content (%)	Atomic C/N
CL 1	−11.55	6.03	47.96	4.41	12.68
CL 2	−11.63	11.00	44.48	5.95	8.80
CL 3	−12.09	10.53	40.53	4.59	10.30
CL 5	−15.52	11.75	46.59	15.24	3.57

and an important cytoskeleton protein, which is widespread in muscle tissue and cells [32, 33]. Myosin is the main unit of myofibrils and is a component of cytoskeleton [34]. The proteomics results further confirm that animal materials such as sinews and/or meat were added to cereal food samples CL 2 and CL 3, which is consistent with the nitrogen isotope results.

According to the results of BLAST searches, the specific peptides of foxtail millet (*S. italica*) were identified



in both cereal samples (CL 2 and CL 3). The sequences in  $\beta$ -amylase specific to barley (*H. vulgare*) were also present in CL 3 (Fig. 6), while barley phytoliths were not found. This lack of barley phytoliths is probably due to the fact that only a small amount of barley flour was added, which is in line with the results of carbon isotope analysis. Furthermore, the specific sequences of myosin were assigned to cattle (*Bos taurus*) and chicken (*Gallus gallus*) in CL 2, which is in accordance with the animal bones that were excavated at the Changle Cemetery [14]. In CL 3, the bovine-specific peptides were equally present for cattle (*Bos taurus*), bison (*Bison bison bison*), yak (*Bos mutus*), water buffalo (*Bubalus bubalis*), and zebu (*Bos indicus*). Zooarchaeological data show that other species of bovine bones have not been found in this region except for cattle. Also, climate and geographic conditions at Changle area are not suitable for water buffalo or yak to live in. Therefore, the animal proteins in CL 3 were probably originated from cattle.

Similarly, cattle are also the most likely origin of the bovine-specific peptides in CL 5, and considering its toughness, CL 5 should come from beef that was rich in tendons. Regarding CL 4, specific peptides come from goats (*Capra hircus*), sheep (*Ovis aries*), European mouflon (*Ovis aries musimon*) and Tibetan antelope (*Pantholops hodgsonii*) were present. Considering the specific geographical environment and zooarchaeological results, CL 4 should come from sheep/goat, and it is the earliest mutton kebab identified in China up to our knowledge.

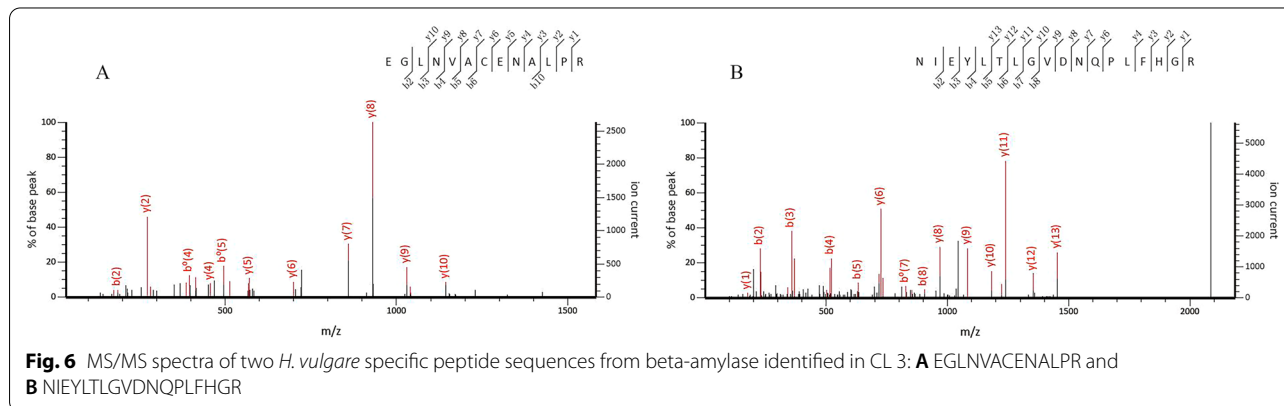
## Discussion

The custom of extravagant consumption in burial ceremonies was prevalent during the Han Dynasty; food-stuffs were one of the typical funeral objects and were commonly found in the Han Tombs [35]. For instance, large amounts of elaborate food remains placed in different dinner-wares were excavated from the Mawangdui Tombs (193–177 BCE) in Changsha, south

China, including various cereals, fruit, cakes, kebabs and other meats [36, 37]. In this study, the specially prepared foods exhumed from Tomb M17 in the Changle Cemetery were placed in certain positions, with cakes placed in front of the coffin, and kebabs and meat strips on the coffin lid. It is likely that they were served as the offerings to the dead at the funeral for higher spiritual enjoyment [38].

Based on the results of phytolith and proteomics analysis, foxtail millet and barley have been identified in the cereal remains. They were made into cakes using different processes: CL 1 was directly made from millet grains, while the other two samples were mainly made from millet flour. The traditional processing techniques utilized for millet crops and wheat/barley were different since the Neolithic time. Foxtail millet has been cultivated in China for at least 8000 years and is usually consumed in grains with boiling or steaming; while wheat and barley, often ground into flour for baking, were domesticated in western Asia and spread eastwards to Ningxia region through the Hexi Corridor approximately 4000 years ago [39–41]. During the Han Dynasty, wheat, barley, foxtail millet and common millet were all staple crops in north-west China. However, wheaten food was not popular in ancient China at that time, probably owing to the local cooking and processing practices, as well as environmental and social factors [42, 43]. The results in this study also show that millet was the principal flour food.

Cooking experiments suggest that these cakes were all processed by baking, which was the popular way of preparing food in western Eurasia [1, 44]. The culinary tradition in eastern China should have been boiling/steaming, which is also reflected in archaeological artefacts such as the water heating pot “Fu” (釜) and steamer “Zeng” (甑). As mentioned earlier, baked cake has also been a traditional food in Xinjiang area for ages. The present study indicates that this dietary custom may have spread eastwards into Ningxia region and that the indigenous people



also mastered grinding-and-baking technologies. It is noteworthy that CL 1, which was the baked cake made from millet grains, represents the integration of diet cultures, with the uptake of baking technique and the maintaining of grain-consuming tradition of millet. The similar-looking cake was also found in the Mawangdui Tomb M1 in south China [36], which would seem to suggest that the cereal grain based-cake was a relatively popular food in the Han Dynasty.

In addition to the cooking style, diet structure was also different between different peoples. Generally, cereal was the staple food for the Han people in central China, while meat and cheese occupied a large portion in the diet of the Hu people, a general term for ancient nomadic people living in north and northwest of China. The results of stable isotope and proteomics analyses show that chicken and/or cattle meat were also added to some of the cakes. The addition of meat could improve the flavour and nutrition of cereals but has been rarely reported in previous studies. This type of cake resembles the ancient food *Hu bing/Shao bing* (烧饼) which was recorded in historical Chinese texts such as *Shi Ming* (释名) and *Qi Min Yao Shu* (齐民要术). According to these texts, *Hu bing/Shao bing* was basically baked cereal flour foodstuff, sometimes mixed with animal fat and minced meat. The findings of our study would stand as the direct evidence of this kind of food in ancient China.

Besides the cattle and chicken meat added in the cake samples, some dried beef and a mutton kebab were also recognized in this study, which indicates a diversity of meat sources. Although kebab is also a traditional Hu food, the scenes of roasting skewered meat were frequently depicted in cooking and banqueting images in the Han tombs of the upper class [45], and more roasting tools such as grills were found during this period [46, 47]. It can be reflected that kebab has also become popular in central China since the Han Dynasty.

Prior to the Han Dynasty, Ningxia region was the settlement for the nomadic people who led a pastoral life [48]. The Han people from inland regions began to migrate into this area during the Western Han period, and promoted the local agricultural economy [14, 49]. This mobility also facilitated the communication among different populations and their food cultures. In this study, the Han immigrants at the Changle Cemetery appear to have maintained some inland dietary characteristics, such as consuming cereal grains directly. Concurrently, the dietary traits of the indigenous Hu people, such as consuming large amounts of meat and the baking/roasting of foodstuffs were also present, suggesting that their diet was also significantly impacted by the customs of Hu people. This cultural communication between the East and West was further accelerated by the opening up of

the Silk Road since *Zhangqian* (张骞), a Chinese envoy from Chang'an (present-day Xi'an), visited central Asia in the late 2nd century BCE, and has continuously enriched Chinese food culture.

## Conclusions

In the present study, we analyzed the food remains excavated from the Changle Cemetery in Ningxia, and found that the cereal foods were baked millet cakes, with animal meat added to some flour foodstuffs; and the meat foods include beef strips and a mutton kebab. These findings suggest that the Han immigrants in Ningxia area had adopted some cooking technologies of the Hu people such as baking and grinding, and transformed them to suit their own ingredients and tastes. This research has promoted our understanding on the dietary customs, food culture and burial rituals in this border area during the Han Dynasty. As an important centre on the Silk Road and a key region of migration, Ningxia played an important role in the constant exchange and integration of diverse cultures. We further suggest that the multi-analytical approach, which could provide comprehensive chemical composition and processing techniques, is of primary importance for the characterization of complex food remains.

## Supplementary information

The online version contains supplementary material available at <https://doi.org/10.1186/s40494-022-00682-w>.

**Additional file 1: Table S1.** Identified proteins and specific peptides in the archaeological food remains from the Changle Cemetery.

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## Authors' contributions

MR and YY designed the research; RFW provided archaeological samples and background; MR performed analyses; MR and YY analyzed data; and all authors helped drafting/revising the manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

## Declarations

### Competing interests

The authors declare that they have no competing interests.

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