INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE
ISSN: 2067-533X Volume 14, Issue 4, October-December 2023: 1405-1416 www.ijcs.ro
DOI: 10.36868/IJCS.2023.04.10

ANALYTICAL STUDY TO DETERMINE THE USE OF ANIMAL RENNET IN THE MANUFACTURE OF ANCIENT EGYPTIAN FOSSIL CHEESE

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Abstract

The recently discovered fossil cheese was carefully analyzed under the current study by using multiple analyzes such as, digital microscopy assessment, FTIR, XRD & SEM in a serious attempt to discover dairy products of Ancient Egyptians furthermore; knowing the type of rennet that was used to coagulate the milk, is it vegetable or animal? To make the results more accurate, fresh cheese samples were prepared from the pastures of the rich Saqqara region according to the old inherited recipes to make a revealing comparison with the archaeological sample then the fresh sample was subjected to accelerated heat- light ageing;the results revealed a striking similarity between the archaeological and the aged fresh cheese where the digital microscope revealed that both samples have the same structure but archeological sample was more fossilized which may be attributed to the survival of Ptahmes's cheese for thousands of years under the burning sands. It should be noted that the analysis of the infrared spectrum revealed accurate and important information about the technology of dairy products in the ancient Egyptians, especially that the authors compared the Ptahmes sample with the aged modern sample and a dried calf rennet sample, to prove or deny the Egyptian's use of calf rennet to coagulate milk, and it was a resounding surprise a presence of a band associated with N-H stretching vibration and hydrogen bands presented in collagen were detected which suggests the existence of helical arrangements of chymosin -solubilized collagen from casein furthermore a matching between the archaeological sample and the dried rennet in the region from 2000 to 4000 cm-^{1} was detected which suggests using the calf rennet in curdling of milk in the ancient Egyptian civilization, the infrared spectraalso confirmed a presence of crystalline residues of fenugreek and red pepper, which are still used until now in storing cheese in upper Egypt.

Keywords: *Chymosin; Dairy products; Calf rennet; Natron salt; Fenugreek*

Introduction

Inside one of the broken pottery jars in the tomb of Ptahmes, high-ranking official under kings Seti I or Ramses II, about 1290−1213 BC. The sample in this study was discovered by The Egyptian mission affiliated to the excavations of the Faculty of Archeology - Cairo University. The tomb of Ptahmes is located 30 kilometers from the Egyptian capital, inside the ancient necropolis of Saqqara, which is replete with many royal and high-ranking official tombs. In 1885, this tomb was first discovered, but with the time the desert sand covered it until it disappeared completely until it was rediscovered again during the excavation season of Cairo University in the winter of 2014. Although seven years have passed since this astonishing discovery, there were no specialized studies on discovered cheese, except for the only study for 2018 [1], through a joint research between the Italian University of Catania and Cairo University.The research paper focused on pathogenic bacterial strains and tracked their genetic impact in milk and cheese furthermore, the paper used GCMS analysis to detect the type of

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milk that used in cheese making, But the research paper did not succeed in determining the exact type of casein [2, 3].The answer was still confused between goat milk or buffalo milk ,which of them had been used to make Ptahmes cheesefurthermore, there was also no indication of the type of clotting factor used; Therefore, the current study aims to answer these questions in addition to the comprehensive examination of this rare piece, which contributes to uncovering the truth absent from the food industries of a civilization that is the oldest among the world's civilizations.

Milk coagulation is the main step for cheese making which had been undertaken by proteolytic enzymes;from cave paintings around 5000 BC was the earliest indication of cheese making; from historical records, there are several references to that enzyme preparations extracts from the stomachs of ruminants was the most enzymes have been used for cheese making,the enzymes extracted from the stomachs of animals were not the only source, but there were also the coagulants from microbes and plants that were used at very early ages. The animal rennet extracted from the stomachs of young calves was the most widely distributed [4, 5] because it contains of big ratio of chymosin (EC 3.4.23.4) the latter is the main responsible enzyme for completing the clotting of milk and turning it into cheese [5-10]. Therefore, the authors resorted to a new, unconventional idea, which is to make aged fresh cheese sample according to the old recipes in upper Egypt and Saqqara region and compare it with the archaeological sample in order to recognizethe essence of Ptahmescheese ; all the recipes that were used in the manufacture of aged fresh samples were based on a field research for the people of Saqqara region and their inherited description since ancient times side by side some writings on the walls of tombs, temples and various sources [11].

By using the infrared spectroscopy, microscopic examination, and X-ray diffraction analysis, the study revealed a number of answers that were stuck in the minds about cheese making in the ancient Egypt. But also it should be noted that The study encountered many difficulties, including: the lack of literature related to the dairy industry in the Egyptian civilization added to fossilization of the archaeological sample, which obstructs the analysis of organic residues of casein. But fortunately, organic casein compounds crystallize over time to form salt crystals that can be analyzed by infrared spectroscopy.

The study aims to prove or disprove the use of calf rennet in milk coagulation in ancient Egyptians, especially since there was confusion among scientists about the nature of rennet at that time. In this sense, two aspects were taken into account: (i) providing a physical and scientific proof of the cheese-making technology in the ancient Egyptian civilization through the careful examination of an archaeological sample of cheese dating from the 19th dynasty and (ii) the answer to the following question: what is the nature of the milk used to make this type of cheese?

Experimental part

Archaeological and freshchees samples

Under this study, a sample of archeological cheese extracted from a jar inside Ptahmes tomb (Figs. 1 and 2) was analyzed in addition to freshbuffalo cheese, the fresh cheese were prepared from the pastures of Saqqara region, by an old woman according to the recipes inherited in the region for generations; the source of milk was buffalo milk only because the ancient heritage refers to the use of buffalo milk due to its high fat, which gives a good cheese for storage, the steps of making cheese was as the following:

i) The curdling process was performedby calf rennet. The calf rennet was added after heating the milk at 50°C at a rate of 1.5cm^3/kg mof milk withstirring well;

ii) The milk has been leaved to curdle for 4 hours, until it turns into a thick gel;

iii) Then the curdled milk was filtered bytraditional mats made of plant sticks;

iv) By using a sharp knife the cheese had been cut into square pieces then it was sprinkled with coarse salt (Halite) and left to dry for two days;

v) The cheese has been storage in the pottery jar with whey cheese, Red Chile Pods, Powder of dry fenugreekand halite salt, and the jar has been closed witha canvas fabric for 1 year in a shady place (Fig. 3).

Fig. 1. (A) Shows the broken pottery jars discovered inside the tomb of Ptahmes, (B) shows that one of these contained a solidified whitish mass (chees)

Fig. 2. Shows the archaeological cheese, we can observe that the cheese turned to poweder under attack of degradation factors via thousand years

Fig. 3. Shows the preperation of modern chees samples: A - modern cheese by coagulating buffalo milk with calf rennet; B - pottery jar where the cheese was stored - jar nozzle closed with a canvas fabric in a technique similar to Ptahmes's jar; C - additions added with the stored cheese: hot pepper cones and ground fenugreek, D and E - the chees after ageing turning to fossilized cheese

An important coincidence was observed, that canvas fabric fragments were found in spout of Ptahmes's jar. Which may indicate that the canvas fabric has been used to seal the jars; which can confirm that the inherited technique in cheese-making in upper Egypt may come back to the Pharaonic times. Accordingly, there is a similarity in principle in the storage in a pottery jar, in addition to the use of fabric to close the jars between Ptahmes's cheese and freshly prepared cheese.

Accelerated heat - light ageing

In order to model the long-term degradation processes in an appropriate time scale, the fresh sample were exposed to heat accelerated-aging. The dry-heat aging at 80±5°C for a month in accelerated ageing chamber. Then the sample subjected to artificial aging by using UV lamp, Xenon light, 5000Watt, 300–600nm for a month.

Digital microscope assessment

For comparison between aged & archeological sample, the digital microscope assessment was under taken by USB Digital microscope (200×) Innovation beyond imagination. SINOTECH Ltd. England.

FTIR spectroscopy analysis

FTIR was used for comparing between aged sample and archeological onesfurthermore a control sample of calf rennet; the means of FTIR spectroscopy recorded by a Nicolet 380 FT-IR with the frequency range of 4000–400cm^{-1}and a resolution of 4cm^{-1}, the data detected in the transmission mode.

SEM

The microscopic investigation was performed to recognize the morphology of archeological and aged modern cheese and comparing the features of the two types. The samples were studied by ESEM, Philips XL 30 at the central lab of the National Research Center (NRC) in Giza, Egypt. Coatingwith gold was required to investigatethe cheesesamples.

X-ray diffraction analysis

The patterns of X-ray diffraction of powder samples were detected using a Philips PW 1840d XRD diffractometer. The instrument was operated at a power of 40kV using a Cu-Kα 36 radiation wavelength 1.54053Å and generator current of 25mA. Mineral phase interpretations were accessed by a match 3 software.

Results and discussion

Visual assessment

Usually, the microstructure of cheese is the spatial arrangement of the casein micelles that join together into clusters and chains to form a viscoelastic protein network throughout which moisture, fat globules, minerals and bacteria are dispersed. Microstructure is one of the major controlling factors of texture (firmness, cohesiveness, elasticity, pastiness, crumbliness) and functional properties of cheese; all of the above is characteristic of modern cheeses, but what is left of this compositionfor archeological cheese?

The micrographs in figure 4A and B shows totally porous surface of archaeological cheese, which can be attributed to the advanced decompositionof fat globules over thousands of years furthermore, the image proven the completely cohesionloss of cheese under the burial temperature. By comparing the microstructure of archeological cheese (Fig. 4A and B) with aged modern cheese (Fig. 4C-D), we can observe a great similarity in terms of color, shape and size, but the pores in archaeological cheese are relatively larger.

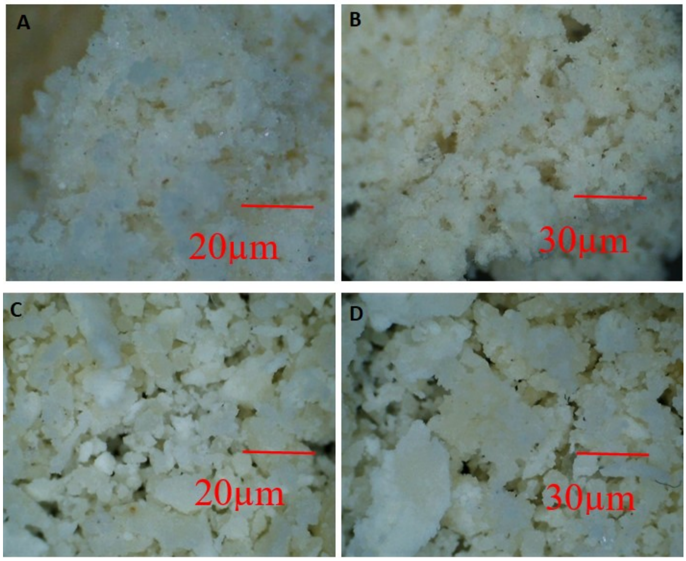

Fig. 4. Shows the microstructure comparing between archeological cheese:
A and B - the aged modern cheese; C and D - We can note the noticeable similarity between the two samples

Fig. 5. Shows the microstructure of dried rennet: a and b - archaeological cheese;
c and d - we can observe brown residues in cheese that could be residues of rennet

FTIR spectroscopy analysis

Figure 6 shows the IR - spectra of archeological and aged modern cheese. In archeological cheese it can be detect a slight N-H stretching band at 3400cm^{-1} (topically band of casein) and symmetric stretching (=CH) at 2830cm^{-1}, those vibration is always higher in buffalo milk compared to goat's milk, therefore, the presence of those vibrations may be a sign of buffalo milk have been used in manufacturing of this cheese, we can observed that asymmetric stretching CH(–CH2) which appears at 2938cm^{-1} was completely lost furthermore, it can be observed a bending CH(–CH2) at wavenumber 1463cm^{-1} and symmetric stretching C=C (amid I) at 1692cm^{-1} [12]; furthermore, no signal was observed about amid II absorptions; the literature stated that structure of protein thought ripening progressiontotally changes ;where the alpha-helix structure decrease dramatically and the beta-sheet structure increase under impact of strengthening of hydrogen bonds that results from hydrolysis of caseins [13-14]. Thus, we can confirm that the ancient Egyptian cheesecoagulatedwith very strong clotting factor such as calf rennet [15]. To confirm the validity of this theory, the authors analyzed the calf rennet by FT-IR (Fig. 7), from comparing between IR of calf rennet and archeological sample we can detect a weak vibration of N-H stretching band in the range 3465cm^{-1} also a amide A band vibration was found at 3076cm^{-1}; from these results itwas clear that amide A band associated with N-H stretching vibration and hydrogen band starching which suggests the existence of herical arrangements of chymosin - solubilized collagen from casein [16-17]. Furthermore, we found notable matching between the archaeological sample and dried calf rennet in the region from 2000 to 4000cm^{-1} which suggestsusing the calf rennet in curdling the milk of Ptahmes's chees.

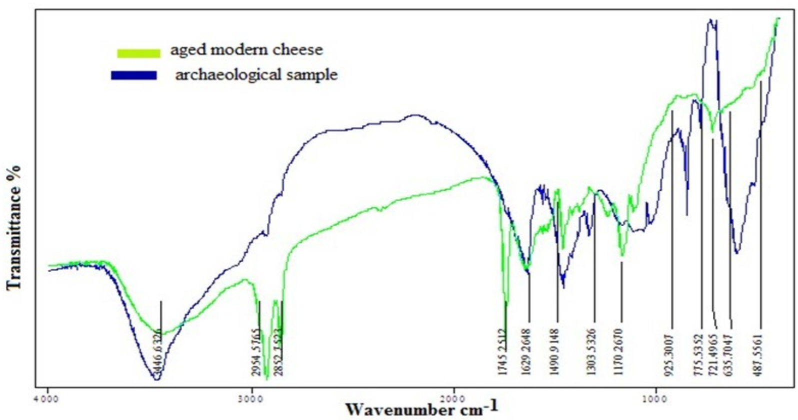

Fig. 6. Shows the spectra of aged modern and archeological cheese

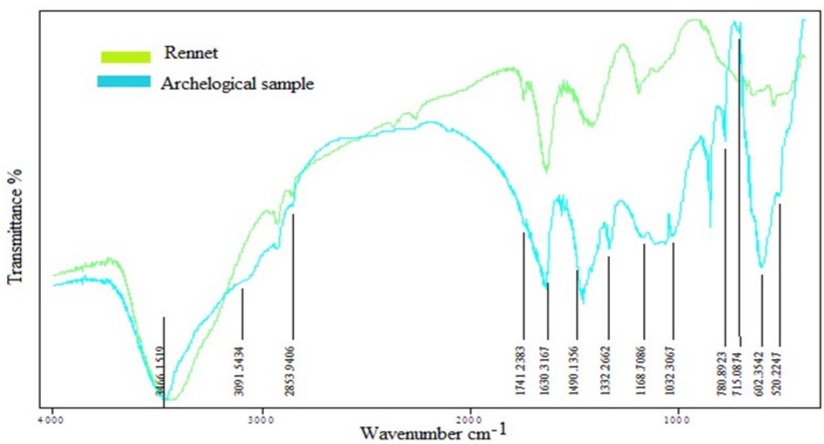

Fig. 7. Shows the spectra of dried calf rennet and archeological cheese

There arealso multiple sharp bandsof Potassium carbonate (K_2CO_3) at 401, 459 and 447cm^{-1}, chemically, buffalo milk has highest fat content. The latter is directly proportional to the potassium ratio, therefore present of these sharp stretching bands suggests that a high-fat milk had been used in Ptahmes's chees, which undermines the theory indicated that goat milk had been used in manufacture of cheese in ancient Egypt. Fantastic results were found by comparing the spectra of aged and archeological samples because the absorption bands at frequencies 1062, 1058 and 598cm^{-1} beside a strong absorbance band at 793cm^{-1}, which is due to phosphate compounds were detect in both samples. The literature found that inorganicparticles of fenugreek presents at these above frequencies [18-19]. Therefore, it could be concluded that the fenugreek had been used in storage Ptahmes's cheese. On the other hand, the frequency at 892cm^{-1} (topically bands of red chili pepper [20]), was observed also in spectrum of Ptahmes's cheese. These fantastic results confirm one thing that the method of storing Ptahmes's chees is the same method used by the current Egyptians in Upper Egypt and villages to preserve their cheeses for long periods, despite the passage of thousands of years, the preservation process remained inherited as it is.

SEM

For a deeper understanding of microscopic examination for cheese samples (archaeological and aged modren sample); the cheesestructure underelectron microscope should be elucidated; *Eino et al.* stated that the animal rennet based cheese characterize a porous, coral-like structure that is almost crystalline in appearance. The protein and fat matrix have predominance of small pores, many less than a micrometer in width since whey may occupy most of these pores. With time the coral-like appearance is largely replaced by a globular network [21].

Furthermore, the literature stated that the cheese structure appears as a porous protein network, with spherical fat globules dispersed throughout. Qualitatively, increasing the concentration of rennet per mass of protein from low to high increase the porosity of the chees also cheeses with a coarser protein network were observed with higher rennet concentrations [22-24]. We can observe the multiple gaps within the structure of the archaeological cheese (Fig. 8) this can be attributed to porous protein network as a result of use the animal rennet in clotting the milk in ancient Egypt.

Furthermore, we can observe a coarser structure network which can be suggested that ancient Egyptian had been used high concentration of calf rennet to obtain very compact structure for their funeral cheese .Furthermore the high concentration of calf rennet decrease the fat ratio in cheese, this ensures that the cheese is preserved for a longer period of time therefore ,we can observed the heterogeneity of archeological sample where it contains both crystalline and amorphous structure (Fig. 8).

Moreover, the fats of cheese were dissolved completely, leave behind a petrified metal structure. On the other hand, we can have observed a porous, coral-like structure that is almost crystalline in in both samples (Fig. 8C and D) but spherical fat globules dispersed is more clear in aged modern samples perhaps the reason is that the accelerated aging has not completely dissolved all the fat of modern cheese. In contrast to the archaeological sample, where fat globules were completely dissolved, as was proven from the previous analysis of FTIR where CH2 stretching at 2900cm^{-1} was completely absent in Ptahmes's cheese.

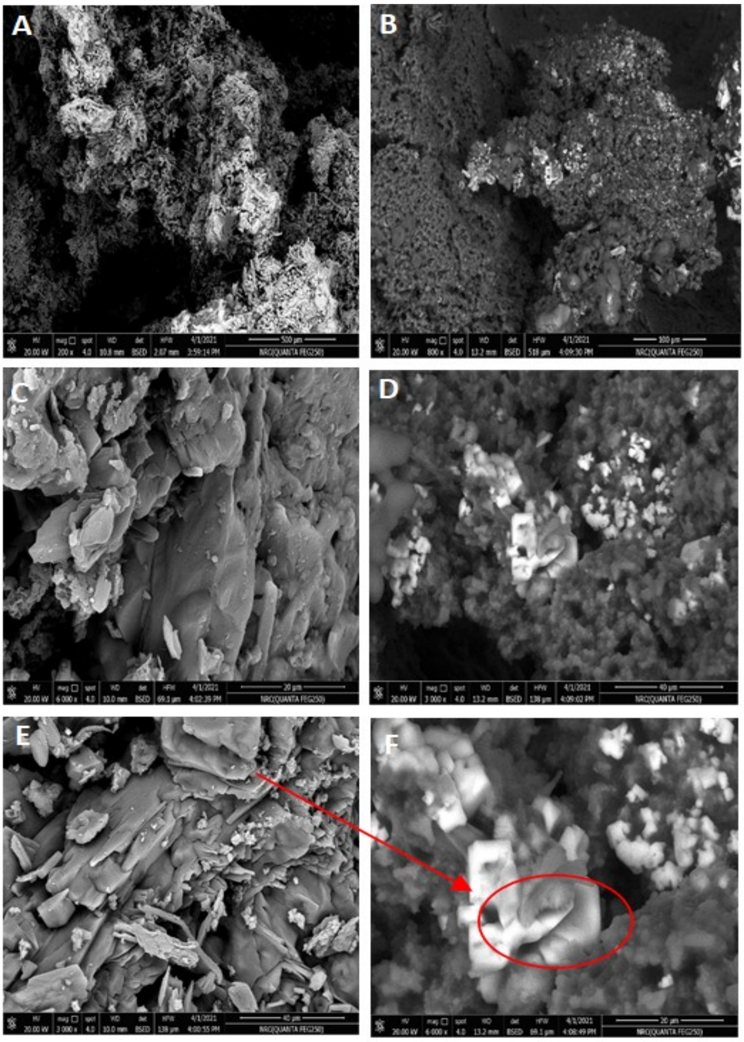

Fig. 8. Showing a comparison between morphological examination of the Archaeological cheese: A, C and E - aged modern cheese; B, D and F - the examination reveals commonalities in the morphology between the two types of cheese, where the A and B appear to be identical except that the structure is completely collapsedin the archaeological sample (A). The stratification of casein ka molecules can also be observed; E and F - but to a widespread degree in archaeological cheese, possibly for lipolysis, which made the remaining casein compounds clear to this extent

XRD analysis

X-ray diffraction analysis was undertaken for dried rennet, aged modern cheese and Ptahmes's cheese to find out the extent of congruence or difference between these samples, which serves the research objective. From figure. 9, we can find that dried rennet contains halite salt with a percentage of 94% in addition to a small ratio of calcite ($CaCO_3$).

Halite Salt enters the rennet industry as a preservative during enzyme extraction processes [25], but it had been used separately in the food industries of the ancient Egyptians? Let's find the answer from the rest of the results. Calcium carbonate crystals in rennet sample could be attributed to crystalline chymosin (Enzyme of rennet) [26-29].

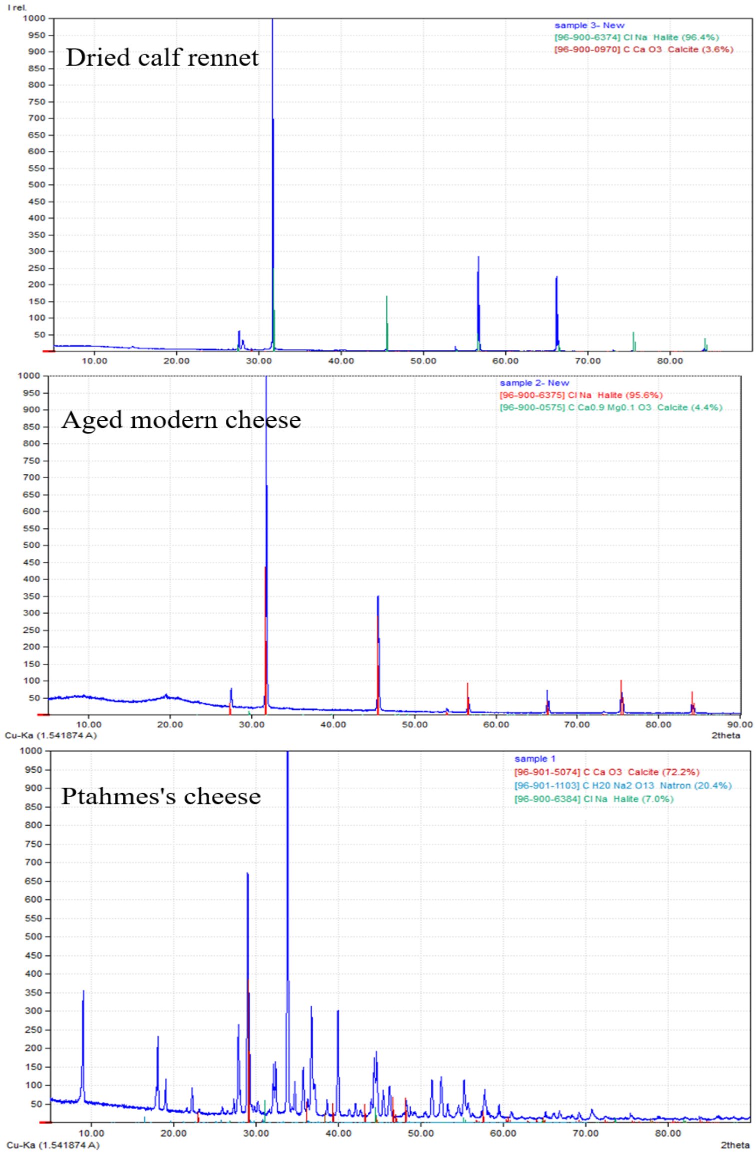

Fig. 9. Shows XRD diagrams of dried rennet, aged cheese and archeological cheese

The result of X-ray diffraction of aged modern cheese showed that halite and calcium carbon ratios were very high as compared with dried rennet because the halite has been used in the storage of modern cheese (see sample preparation section). Furthermore, the high ratio of calcium carbon attarabtied to crystalline chymosin in fresh rennet which used in making the modern cheese; in Ptahmes's cheese XRD pattern, we can observe that the ratio of sodium chloride is very low, as if they were impurities, but Natron ratio is very high, reaching about 20.4% which suggests that the ancient Egyptians used Natron in the cheese-making. But what is the source of haliteratio that found in the archaeological sample?

The answer could be the chemical composition of Natron, yes, Natron is mixture of sodium carbonate decahydrate (Na_2CO_3•$10H_2O$, a kind of soda ash) and around 17% sodium bicarbonate ($NaHCO_3$) along with small quantities of sodium chloride and sodium sulfate; the high ratio of calcium carbonate in the archaeological sample, it may be due to the interaction of calcium oxide in cheese with carbon dioxide in the closed atmosphere of the tomb to producecalcium carbonate [30] or may be due to soil particlesespecially that burial soil in this region is richof calcium carbonate. On the other hand, this ratio could be attributed to the residues of crystalline chymosin (rennet enzyme).

Conclusions

The current studyuncovered a lot of secrets of Ptahmes's cheese, this can be summarized in the following points:

- The buffalo milk coagulated with animal calf rennet had been used in manufacture of Ptahmes's cheese where chymosin residueswere detected by infrared spectroscopy furthermore SEM examination confirmed that high-fat milk had been used, such as buffalo and not goats in this cheese
- XDR result confirmed that the ancient Egyptians used Natron salt in the dairy industry.
- The significance of the study did not only contribute to revealing these facts, but alsorevealed that there are a number of methods and technology that had been used in ancient Egypt and are still used today, whereasthere was a great similarity between the sample prepared according to the heritage of the ancient people of Saqqara and the archaeological sample.
- The residues of fenugreek and red chili pepper were also detected in the archaeological sample, and they are the same ingredients that are still used in storing cheese by the original people of Saqqara.
- The study is a promising stepin this field and abridge between the inherited heritage and one of oldest ancient civilization in the world, but there is still a long way to go to reach the full information and we need more studies and comparisons in this distinguished field.

Acknowledgements

We thank to Professor Ola El-Aguizy Head of the Cairo University Excavation Mission, Faculty of Archaeology, Cairo University 12613 Giza, Egypt for her assistant for collection of archeological samples

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Received: September 25, 2022
Accepted: September 14, 2023