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RESEARCH ARTICLE

PHASE ANALYSIS AND MICROSCOPIC ANALYSIS OF THE SHELL OF CALCINED OYSTER WITH MINERAL CHINESE MEDICINE

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ABSTRACT

Objective: To explore the composition and microstructure of oyster shell. Methods: The composition of calcined oyster shell was analyzed by powder X-ray diffraction, and the microstructure of calcined oyster shell was observed by scanning electron microscope. Results: The XRD patterns of calcined oyster were consistent with the characteristic peaks of calcium carbonate PDF standard card 88-1807, silicon dioxide PDF standard card 85-0794 and calcium hydroxide PDF standard card 87-0673. Conclusion: The main component of calcined oyster shell is calcium carbonate, and contains trace amount of silicon dioxide and calcium hydroxide.

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INTRODUCTION

Oysters, aliases: clams, ancient oysters, Zuogu oysters, clams, oyster houses, oysters, oyster shells, left shells, sea oyster shells, sea oyster skins, belong to the Mollusca (Bivalvia) Pteriomorphia (Ostreoida) oyster general family (Ostreoidea), the distribution range is global, there are more than 100 species in the world, there are more than 20 species in the coastal areas of China (Li Chaomei, 2000). The main medicinal oysters in modern China are the crassostrea gigas Thunberg. Ostrea rivularis Gould. Or Dalian Bay Oyster Ostream Alienanensis Crosse. Such as (Yang Yun, 2015). As a kind of high quality Marine cultivated shellfish, oyster not only has the edible value of delicious meat, but also its shell can be used as medicine and has high medicinal value. As the largest cultivated shellfish in the world, oyster is one of the important Marine living resources available to human beings. Oyster has a delicious taste and high nutritional value. Known as "undersea milk", oyster meat contains a variety of amino acids, glycogen, a large number of active trace elements and small molecular compounds, and its shell contains a large amount of calcium carbonate (Li Longfei, 2014). Oyster is popular among consumers for its delicious taste and rich nutrition. However, when people eat oyster shells, a large number of them are discarded as garbage, which not only wastes resources, but also causes environmental pollution.

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In fact, more than 90% calcium carbonate in oyster shells is a valuable resource, which can be used in many fields, such as medicine, food health care and making various additives. Application in pharmaceutical value: oyster shell is a kind of traditional Chinese medicine, pharmacological researchers in our country on the basis of the traditional medical experience using the modern research techniques of oyster shells made in-depth study, found that its effectiveness, including liver yangxin NingZhi, flat extinguish wind and liver latent Yang, phlegm cough, eliminate the production of body fluid to internal (You Donghong, 2010). Oyster shell and keel both contain a large amount of calcium, rich trace elements and a variety of amino acids, and the types are similar. Therefore, oyster shell may be used as a substitute medicine for keel to make up for the shortage of keel resources (Chen Yuzhi, 1999). Oyster shell is the part of oyster medicine, salty slightly cold, return to the liver, gallbladder, kidney. Function and indications: it can smooth liver latent Yang, astringent and astringent, soft and firm and diffuse knot, calm and calm (Bin Song, 2019), enhance immunity, anti-fatigue, antiviral, protect liver, hypoglycemia, anti-tumor, anti-oxidation, antibacterial and other effects. It is applicable to convulsion, insomnia, dizziness, tinnitus, scrofula, scrofula and scrofula. Calcined oyster is astringent, astringent and astringent, and can produce acid and relieve pain. It is used for self-perspiration, spermatorrhea and slurries, leakage under the belt, stomachache and acid swallowing, etc. The morphological characteristics of three kinds of oysters in China are as follows: Two pieces of shell near the river oyster, firm and thick, round, oval or triangular in shape.

Left shell attached, large and thick. The right shell is slightly flat, smaller than the left shell, the surface is ringed with very thin and straight yellowish-brown or purplish-brown scales; Individual of 1 ~ 2 years, the scales are flat, thin, brittle, and sometimes free; Individuals of 2 to several years, scales flat, sometimes undulating into weak water waves at the trailing edge; Individual with many years of growth, overlapping layers of scales, hard and thick as stone. The surface of the shell is gray, green, purple, brown and other colors, the inside is white, the edge is gray purple. The ligaments are purplish black, the incisors of the obturator shell are large, pale yellow, mostly oval or nephritic, located in the middle dorsal. Foot degenerate, without foot filaments. Living in rivers, rivers and sea of people. Omnivorous, feeding on tiny plankton. It is distributed along the coast of China. Guangdong, Fujian, Shandong coastal, and breeding. 2.Crassostrea gigas shell large, thick, oblong, dorsal abdomen almost parallel, the general shell length is three times higher than the shell. Left shell attachment. The right shell is flat such as the cover, scales ring, show corrugative shape, arrange sparsely, the level is very few. The surface of the shell is mauve, off-white or tawny. The inner surface of the shell is porcelain white. Closed shell muscle scar horseshoe-shaped, brownish yellow, dorsal in posterior part of shell. Left shell concave, scales thicker than right shell. The fleshy part is soft, the gills are straight, do not bend to the back Angle. All along the coast of our country. It is a good breed for estuaries and inner bays.

3.The shell of The Dalian Bay oyster is large, medium thick, extending from front to back, with the top to the rear gradually expanding into an approximate triangle. Left shell attachment. Right shell surface scales undulate into water waves, not as flat as near the river oyster, radiation rib is not obvious. The shell surface is light yellow; The inside of the shell is white. Closed shell muscle scar white or purple, located in the back square. The fleshy part is elongated, the gills extend from the front to the rear center, with little bending. It is distributed along the northern coast (Li Chaomei, 2000). Drug characters: irregular ovoid, triangular or oblong shells, varying in size, usually 10 ~ 30 cm long, 5 ~ 10 cm wide, 1 ~ 3 cm thick; Appearance gray, light gray-brown or gray-blue, layered, and has a curved rough layer. The inner surface of the shell is mostly opalescent, smooth and glossy, with transverse striations at the base, lackluster, and wavy laminations at the edges. The left shell is thicker and larger than the right shell, not flat, shell outside often have conch, moss and other attachment, the surface and often have a hole, there are small shells; The right shell is thin and small, relatively flat. Hard, not easily broken, white cross section, layer. No gas, slightly salty taste. A large, neat and clean inside is preferred.

Zhao Juan et al. believed that the oyster shell is composed of the outermost proteinaceous cuticle, the middle prismatic layer and the inner pearl layer. The prismatic layer is the main structural part of the oyster shell, which is a lamellar structure interwoven with calcium fibers. The material composition of oyster shell can be mainly divided into two categories, one is the organic part that regulates and frames, and the other is the inorganic part (Zhao Juan, 2015). The organic part of oyster shell is mainly composed of protein, glycoprotein and polysaccharide, and also contains 17 amino acids such as glycine, cystine and methionine, among which the content of aspartic acid is up to 1800mg/kg. In the inorganic part, the content of calcium carbonate accounts for more than 90%, in which the content of calcium element accounts for (39.78± 0.23) %, and the content of strontium in trace elements is as high as 2631mg/kg. In addition, it contains 24 trace elements such as iron, zinc and selenium [(9)].Iron is a component of hemoglobin, myoglobin and cytochrome. It plays a very important role in the process of human metabolism. It is directly involved in the transport and storage of oxygen. Iron deficiency in the human body will lead to decreased immune function, anemia, fatigue, reduced resistance, stunted growth and so on. Manganese is involved in the synthesis and activation of many enzymes, the metabolism of sugar and fat, the acceleration of protein, vitamin C, vitamin B synthesis, catalytic hematopoietic function, endocrine regulation, is an essential element to maintain normal bone structure. Zinc element has the function of clearing heat, cooling blood, reducing inflammation and generating muscle. The content of zinc is higher in Chinese medicinal materials which have the functions of clearing heat, calming liver and extinguishing wind, promoting blood circulation and reinforcing Yang. Copper plays an important role in hematopoietic growth, cell growth, the activity of some enzymes and endocrine functions, but excessive intake will damage the morphological structure of tissues and organs, interfere with the transfer function of cell membranes and important enzyme system activities [(10)]. Molybdenum is a component of the enzyme that catalyzes the last step of purine conversion to uric acid. It is also involved in the metabolism of toxic aldehydes and is essential for energy transfer in cells as well as the function of certain intestinal enzymes. Chromium is an element essential for the use of insulin during glucose metabolism, especially for promoting the synthesis of insulin from simple substances into fat. Magnesium can activate cholinesterase, choline acetylase, phosphatase, etc. Magnesium ion has an inhibitory effect on the central nervous system, which is consistent with the important calming effect of oyster. Manganese is also an essential trace element for life.

It can activate more than 100 enzymes and plays a very important role in energy metabolism, protein metabolism and mucopolysaccharide formation. Manganese itself is a component of a variety of enzymatic activity centers. It has many biological functions, such as promoting growth and development, ensuring normal endocrine function, participating in human bone hematopoiesis, and promoting intracellular fat oxidation (Yan Xingli, 2009). Organic matter is divided into soluble organic matter and insoluble organic matter, and its content varies with shellfish species and growth period. Its content varies with shell species and growth period, generally accounting for 0.01% ~ 10% of the dry mass of shell, among which the soluble organic matter content is even less, accounting for 0.03% ~ 5%. After oyster forging, the contents of trace elements except lead and manganese all increased to different degrees, and the amount of zinc was increased. Therefore, when oyster shells are used as Chinese medicinal materials, the required efficacy is different and the processing methods are different (Yuan Xi-fan, 1991). The application of oyster shell in health care mainly includes the following two aspects: Preparation of active ionized calcium .As a drug carrier. Oyster shell is mainly composed of prismatic layer, due to its special physical structure and the oyster shell contains a lot of lamellar 2 ~ 10 microns of microporous structure and biological activity of amino polysaccharide and protein, processing can produce a variety of different functions and, if the cavity structure, has the strong ability of adsorption, inclusion and catalytic decomposition, and so on. Bone replacement biomimetic materials are prepared from oyster shells. The formation of oyster shell is highly similar to the formation of coral and bone salt deposition in human body, which will provide a theoretical basis for the development of oyster shell with rich resources into a bone tissue engineering repair material with good performance. Agricultural applications: Preparation of soil conditioners from oyster shells. Natural slow-release fertilizer and calcium fertilizer were prepared from oyster shells. Application in light industry: Preparation of food preservatives and preservatives from oyster shells. In recent years, it has been found that the shells of most Marine shellfish have obvious antiseptic effect after calcined at high temperature. Among them, the calcination of oyster shell has the strongest antiseptic effect, which is obviously better than the analysis of pure calcium oxide. Preparation of cement additive and coating additive from oyster shell. Oyster shells contain 95% calcium carbonate, while ordinary limestone contains 80%. Therefore, oyster shells can replace limestone to make shell cement. Adding 10% oyster shell powder into long-term high-strength concrete can significantly improve the freeze-thaw resistance and water penetration resistance of concrete. Used for sewage purification treatment. There are numerous interconnected pores in oyster shell, which can produce a variety of pore structures with different functions after treatment, so that it has strong adsorption capacity, exchange capacity and catalytic decomposition, and can well absorb the effective components in fertilizers and drugs, and can be used as a good adsorbent to treat sewage. In addition, oyster shells are also used as flame retardant plastic materials, adsorption clarifiers, feed ingredients and so on. As well as the classification of oyster traits and the identification of morphological characteristics (Miao Jianyin, 2012). Oyster shell by-products accompanying oysters have caused social and environmental problems due to lack of treatment methods and technologies. In order to solve these problems and take advantage of the possibility of oyster shell as cheap material, various research and environmental protection methods are being carried out at present (Sustainable Management of Oyster Shell By-Products and Recent Research Techniques, 2018). In medicine, Xiao Meifang, Lin Luan, Chen Hong Pei and others studied the anti-fatigue effect of oyster polypeptide (OP) and its regulation on intestinal flora in mice (Xiao Meifang, 2020). Ahmed S A,Gibriel Abdullah A Y and others studied the development and evaluation of oyster shell fortified diet formula for preventing and treating osteoporosis (Ahmed, 2015). In this paper, the composition of calcined oyster shell was analyzed by X-ray diffraction analysis, and the microstructure of calcined oyster shell was observed by scanning electron microscope at different times.

Experimental

Materials and instruments

Oyster (from Guangxi Guigang Lvzhiyuan Breeding Development Co., Ltd.). Forging oysters: using the Ming forging method as prescribed in the Pharmacopoeia (a method that does not isolate the air during drug forging);Intelligent High Power SmartLab X-ray Diffractometer 2006 (Japan Science Corporation),scanning the range of 5 ~ 100 °, scanning speed is 4 ° / min, step 0.02 °, each time 3 s, gamma ray source using copper target Cu K alpha 0.154014 nm wavelength, 40 kv tube voltage, tube flow 40 ma current, divergence slit 0.5 °, scattering slit 0.5 °, receiving slit 0.3 mm.Scanning electron microscope SU5000 (Hitachi Japan).

Experimental steps: Sample preparation: take 25g clean pieces in muffle furnace built in crucible, heat to 750℃ for 1h, forge until red, take them out and let them cool. Crushing and sieving with 300 mesh sieve for later use.Experimental operation: Put the calcined oyster powder sample into the sample slot of X-ray diffractometer. During the collection of X-ray powder diffraction data, the surface of oyster sample shall be kept flat. At the same time, in order to eliminate stress and select orientation, the measured sample shall be ground into powder less than 30 m, and external force shall be avoided as far as possible during the loading process. The microstructure of calcined oyster fragments was observed under scanning electron microscope (SEM) at different multiples.

RESULTS AND DISCUSSION

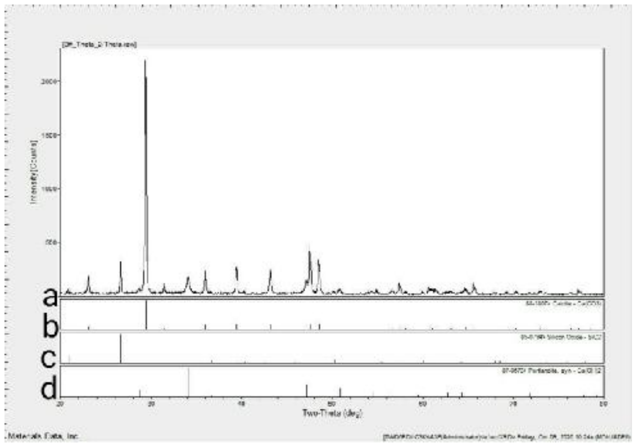

Figure 1. XRD pattern of calcined oyster（$2\theta^{o}$）

Phase analysis of samples: (A: XRD pattern of the sample; B: Calcium carbonate PDF standard card 88-1807; C: Silicon dioxide PDF standard card 85-0794; D: Calcium hydroxide PDF standard Card 87-0673). X-ray powder diffraction method was used for phase analysis. Figure 1 is the XRD pattern of the calcined oyster powder sample. The comparison between Figure 1A and Figure 1B shows that the nine obvious peaks of the calcium carbonate standard are corresponding in the calcined oyster powder sample, and the scanning Angle and intensity are 23.12°(178) respectively. 29.44 ° (2196); 31.46 ° (104); 36.0 ° (248); 39.46 ° (265); 43.16 ° (228); 47.52 ° (466); 48.52 ° (328); 57.44 ° (114); Thus, the main component of calcined oyster is calcium carbonate (CaCO3). According to the comparison between Fig.1A and Fig.1C, the five obvious characteristic peaks of silica standard have obvious corresponding relationships in the XRD pattern of the calcined oyster powder sample, and the scanning Angle and intensity are 20.86°(54) respectively. 26.68° (318); 36.48° (25); 39.46° (265); 50.14 ° (36). It is concluded that the calcined oyster contains silicon dioxide. According to the comparison between Figure 1A and Figure 1D, the five obvious characteristic peaks in the calcium hydroxide standard have obvious corresponding relationships in the XRD pattern of the calcined oyster powder sample, and the scanning Angle and intensity are 28.26°(22) respectively. 34.1 ° (173); 47.52 ° (466); 50.84 ° (63); 54.22 ° (20). It is concluded that calcined oyster contains calcium hydroxide.

Scanning electron microscope (SEM) analysis: SEM has a high magnification ratio, which can be adjusted continuously between 20-200000 times.

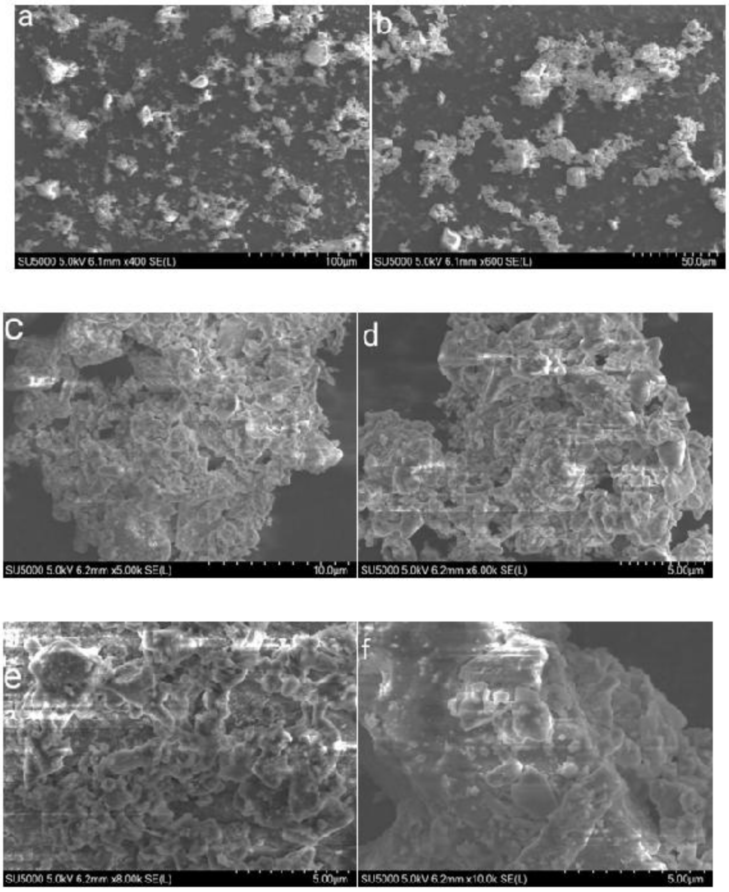

Fig. 2. SEM images of calcined oyster (2a: magnification 400 times; 2b: Magnification 600 times; 2c: magnification 5000 times; 2d: magnification 6000 times; 2e: magnification 8000 times; 2f: magnification10000times）

It has a great depth of field, a large field of vision and stereoscopic image, and can directly observe the fine structure of various samples on the uneven surface. The sample preparation is simple. Fig. 2a shows the calcined oyster fragments amplified by 400 times. Under this magnification, it can be seen that the calcined oyster has more irregular and unevenly distributed products on the surface, including several prominent products with bright colors and sizes ranging from 20 to 40 m.Fig. 2b The calcined oyster fragment is amplified by 600 times, and it can be observed that there are regular projections in about 50% of the surface area, which are more clustered and up to 100 m in length, and there are holes in the spatial structure of the projections.

Fig. 2c shows the scanning electron microscope (SEM) of the calcined oyster fragments magnified by 5000 times. Under this magnification, it can be seen that the microstructures of the projections are multi-layered and irregular, with many small holes in the spatial structure.Fig.2d shows the scanning electron microscope (SEM) of calcined oyster fragments at a magnification of 6000 times. Under this magnification, it can be seen that the surface of oyster shell protruded by many small particles with the size of about 2 m. The shape of the particles is irregular, the layers are not distinct, and there are many gaps in the spatial structure. Fig. 2e is the 8000 times Figure of calcined oyster fragments under SCANNING electron microscope. At this multiple, it can be seen that the structure on the most surface of oyster shell is adherent to each other, and there are many particles less than 0.5 m attached at the bottom layer.Fig.2f shows the scanning electron microscope (SEM) of calcined oyster fragments magnified by 10000 times. It can be seen that the protruded part on the surface of oyster shell is attached to many small particles less than 0.5 m, with a small number of large particles, which adhere to each other to form a spatial structure. The spatial structure is not closely arranged, and there are many small holes.

Conclusion

X-ray diffraction analysis method is applied to study the composition of calcined oyster shell, which has the advantages of rapid, simple, stable pattern, strong specificity of fingerprint and large information, small amount of samples required and no damage to the samples under test. The calcined oyster contains calcium carbonate, silicon dioxide and calcium hydroxide after the above experimental analysis. By scanning electron microscope observation, the microscopic structure of calcined oyster shell can be seen more intuitively, which is of great assistance to the identification and identification of advantages and disadvantages.

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