Role of the Copper Ion in Pidan Processing

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Abstract: The aim of the present study was to investigate the role of the copper sulfate in pidan processing using a novel technology - pressure technology. First, we used scanning electron microscope (SEM) to observe their eggshell microcosmic structure, especially for the pores on the eggshell of duck egg. In order to assay the copper content in duck eggshell and the preserved eggshell, atomic absorption spectrometry (AAS) was used. Results showed that there were many pores on the eggshell of duck egg. The surface of duck egg after acid pretreatment was rough. Besides, there were some particles around the pores on the preserved eggshell. The content of copper in the preserved eggshell was 77.97 μg/g, while the content of copper in the duck eggshell was 28.04 μg/g.
Keywords: preserved egg; copper ion; scanning electron microscope; atomic absorption spectrometry.

1. Introduction

Preserved egg, which is also known as "pidan" or "thousand-year-old eggs", is preferred by most people in China and Southeast Asian countries [1]. Generally, preserved egg can be made by salting fresh eggs in chemical solution comprising salt, sodium hydroxide, black tea powder and copper sulfate for about 4–6 weeks [2-4]. Sodium hydroxide enters the egg through the pores and the semipermeable membrane, contacts the egg proteins, and hydrolyzes the proteins into polypeptides and amino acids. During this action, the albumen liquefies first and then coagulates, forming a gel with certain elasticity. The sodium hydroxide continually diffuses into the yolk. The appearance of pidan becomes a semitransparent tea-brown color, and the yolk is solid or semisolid with a dark-green color. Pidan has an extremely long shelf life and a pleasant, fragrant taste [5]. Traditionally, after salted, ripening is needed to obtain the characteristic mild flavor and aroma [6].

Traditional pickling methods of pidan have disadvantages of long time, uneven quality and complicated processes. So we developed a novel method - pressure method - to pickle pidan. Pidan pickled by pressure technology are not only reduced the pickling period to 7 days, but also shortened processing cycle and brings about enormous economic benefits.

In recent years, scientists have studied the principles of traditional pidan making. PbO is necessary for making pidan of soft yolk in traditional processing. But lead is a poisonous element. Pb accumulating in the human body can damage the nervous system, blood-producing organs, kidneys, and immune system. Currently, scientists are doing much research to determine the principal function of Pb in pidan processing. Other materials such as copper sulfate have been used in pidan processing as a PbO replacement. But the function of copper ion is still in doubt during the processing of making preserved egg. So in our study, we did some research the by scanning electron microscope and atomic absorption spectrometry to study it.

2. Materials and methods

2.1 Chemicals and reagents

Collected duck eggs, which weighed 67 to 74 g, were purchased from a local market in Tianjin City. All cracked or thin-shelled eggs were discarded. Nitric acid, perchloric acid, citric acid, copper standard solutions were analytical reagent.

2.2 Pressure equipment

Duck eggs were pickled in pressure equipment which was custom designed for our laboratory (Fig 1). This equipment consists of the following five basic components: (1) pickling tank; (2) UV sterilizer; (3) duplex filters; (4) PLC control box; (5) pressure pumping system.

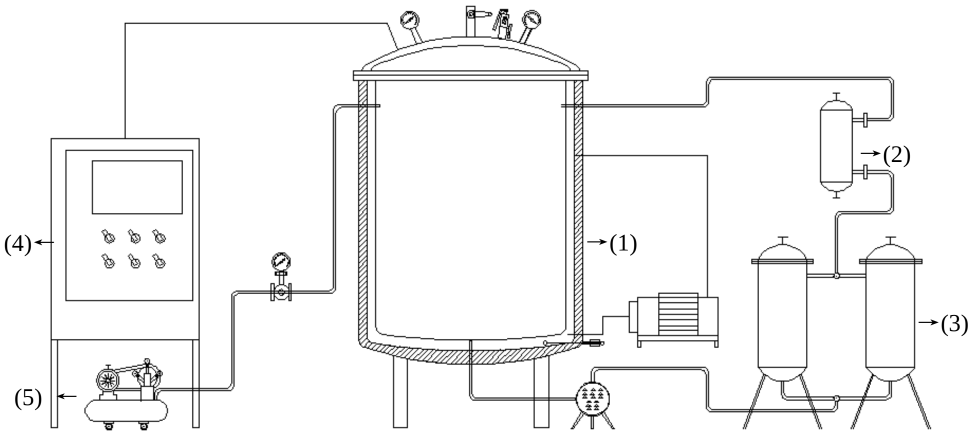


Fig.1 Pressure equipment

2.3 Sample processing

Two groups of duck egg were prepared, one group was pickled directly, and the other was treated by 5% citric acid (ten minutes) before pickling. Duck eggs were pickled 7 days in pressure equipment, and temperature 23℃, pressure 0.10MPa, retention time 24h each day were applied to pickle eggs for 6 days. Then remove all the eggs to thermostatic incubator (23℃) for its ripening period. Three pidan were chosen randomly, and the samples of eggshells were taken.

2.4 Determination of scanning electron microscope

The eggshell microcosmic structure was observed by N. R. King and D. S. Robinso [7]. The eggshell microcosmic structure was measured by the following procedures: the samples of eggshell fragments were taken from freshly broken duck eggs, rinsed in distilled water to remove organic matter and debris, then dried [8]. The samples must be made for right size by tweezers and the samples of cross-sectional should be made by natural fracture. The samples of shell fragments were then attached to sample table with a kind of special double sided adhesive prior to gold coating. And it would be studied in the SEM directly without further examined.

2.5 Determination of Atomic Absorption Spectrometry

For the open digestion method, the following procedure was applied. The samples of eggshell were taken from duck eggs, rinsed in distilled water to remove organic matter and debris, and then dried. Samples were weighed precisely 1.000 g in a digestion flask of 250 milliliter. Then 10 ml concentrated nitric acid and 2 ml concentrated perchloric acid was added and maintained for 12 hours at environmental conditions. The mixture was then heated on the electric heating plate to speed up the digestion until the mixture became colorless transparent liquid [9-10]. And the digestion flask had brown gas during the heating. When the samples were well digested, we should open the lid until the liquid in the digestion flask close to 2 ~ 3 milliliter. Then, the mixture was cooled to room temperature and the resulting solution was transferred into a 50 ml volumetric flask by washing the interior of the flask with small portions of water, and the solution was diluted to the mark. Meanwhile, we should set a blank control group. All solutions were made with deionized water. Copper standard solutions were prepared after successive dilutions of the metals standard stock solutions of 1000 mg/L.

Instrument conditions：Atomic absorption was measured on spectrometer provided with a heated graphite atomizer and hollow cathode lamps for the elements Cu. Lamp current: 7.5 mA, wave length: 285.2 nm, slit width: 1.3 nm and gas flow was 1.8/min.

Standard curve: The standard curves were constructed with 5 points using solutions of the copper standards (0.1 μg/mL, 0.2 μg/mL, 0.3 μg/mL, 0.4 μg/mL, 0.5 μg/mL). The absorbance of copper standard solutions was measured, then draw standard curve, and used the concentration as the abscissa and absorbance as a horizontal coordinate.

Determination of copper: Take 10 microliter sample to measure its absorbance. Then the concentration of sample was calculated through the standard curve.

Formula: The calculation formula of copper content in the sample:

$$X = \frac{(c - c_0) \times V}{m}$$

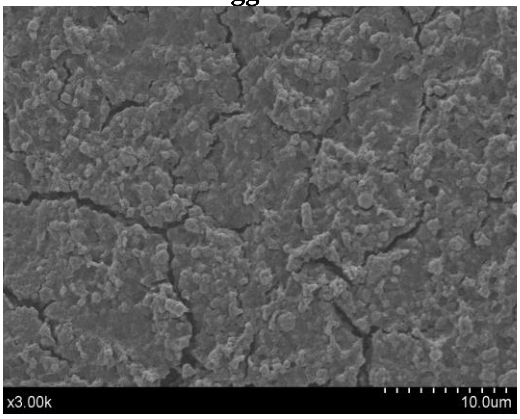
X: the copper content in the sample, mg/g; c: the copper content in the sample solution, mg/mL; c_0: the copper content in the blank group solution, mg/mL; V: constant volume, mL; m: sample weight, g.

2.6 Statistical analysis

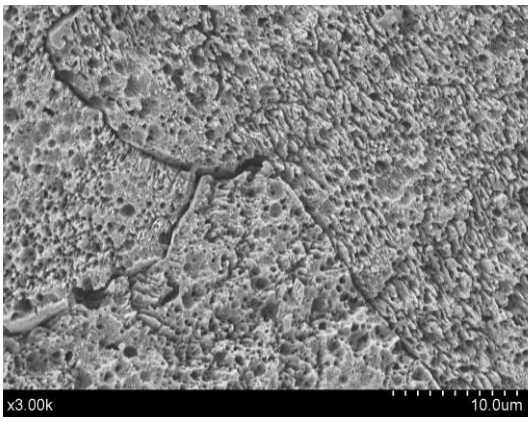
Simples were conducted in triplicate. A randomized design with three replications was used throughout the study. Data were presented as mean values with SD. Statistical analysis was carried out using the software SPSS17.0.

3. Results and discussion

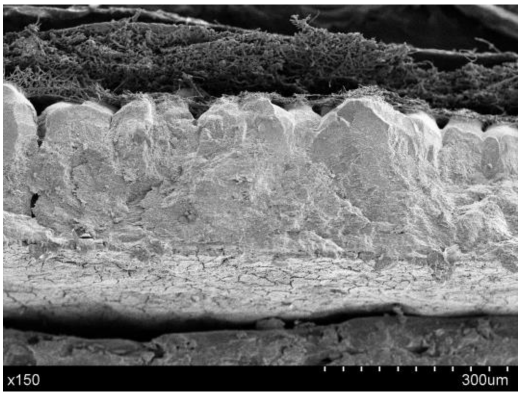
3.1 Determination of eggshell microcosmic structure



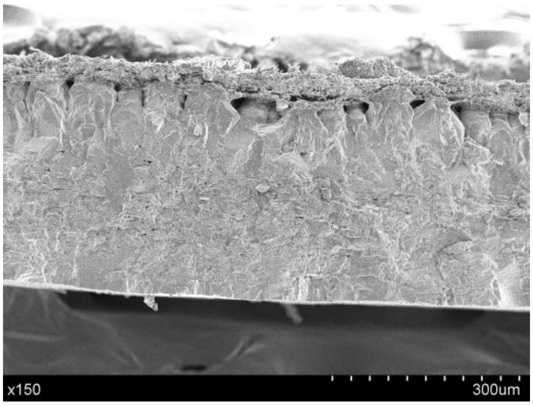
a. The top surface of eggshell×3000



b. The top surface of eggshell after acid pretreatment×3000



c. The transverse section of eggshell×150



d. The transverse section of eggshell after acid pretreatment×3000

Fig.2 The surface of duck eggshell

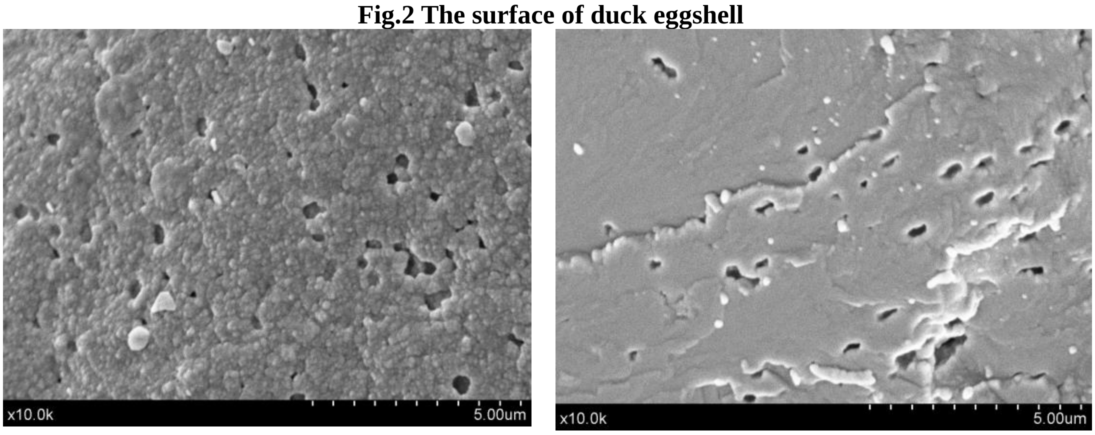


Fig.3 The surface of preserved eggshell after acid pretreatment×10000

From figure 2a, there were many crevice and pores on the eggshell of duck eggs, which were the connection channel of the egg inside and outside. After acid pretreatment, the surface of duck egg was rough (Fig. 2b). And the thickness of eggshell was reduced (Fig. 2c, Fig. 2d). Egg shells are composed of calcium carbonate (CaCO3), known as calcite, which is the more stable polymorph at room temperature [11-12]. Acid can break apart the solid calcium carbonate crystals that make up the eggshell into their calcium and carbonate parts [13]. This reaction caused the decrease in eggshell thickness [14]. Eggshell thickness of less than 0.2 mm was observed when soaking the egg in 0.1 M hydrochloric acid for 30 min [15].

From the figure 3, there were some particles around the pores of preserved eggshell, which reduced the size of the eggshell pores. The explanation was as follows: when sodium hydroxide migrated into the eggshell, the pH of the protein was higher than the isoelectric point. The protein was thus negatively charged. Then the carboxyl group of the protein combined with copper ion, destroying the protein structure while enhancing the protein gelation and decreasing the diffusion of sodium hydroxide. Regarding to the function of copper ion, some scientists think that Cu^{2+} combines with S^{2-} (from protein hydrolysis) to produce black-colored copper sulfide (CuS) [16]. However, Zhang Wenli find that if Cu^{2+} and S^{2-} are separated by an eggshell, they produce copper with trace amount of CuS on the surface of the eggshell at the side of copper chloride solution within one week. Such an unexpected phenomenon strongly indicated that Cu^{2+} is able to be reduced into metal copper when S^{2-} diffuses throughout the porous eggshell [17].These process produced sediments inside and outside of the eggshell. The sediments existed inside and outside of the eggshell and reduced the size of the eggshell pores.

Controlling the concentration of sodium hydroxide inside the egg in late fermentation was very important for making good-quality pidan. High sodium hydroxide content would result in re-liquefaction, and the final product was not typical pidan.

3.2 Determination of the copper content by AAS

There was literature showed that particles on preserved eggshell surface were relevant to heavy metal. And the results of copper content of duck eggshell and preserved eggshell by AAS were showed in table 1.

Table.1 AAS determination of eggshell

Metal	Duck eggshell	Duck eggshell after acid pretreatment	Preserved eggshell
Cu（μg/g）	28.04±2.17^{b}	5.06±0.68^{c}	77.97±4.42^{a}

Different letters in the same line indicate the significant differences ($p<0.05$).

As shown in the table 1, the content of copper in the preserved eggshell was the highest, while the content of copper in the duck eggshell after acid pretreatment was the lowest. It might be due to the copper ion in the outer of eggshell was more than inside. After the citric acid leaching the duck egg, the external copper ion dissolved in the acid, which leaded to the copper content in the duck eggshell after acid pretreatment was lower than others.

During the pickling of preserved egg, protein produced hydrogen sulfide gas due to alkali denaturation. Then copper ion combined with sulfur ion to produce sediments. The sediments exited inside and outside of the eggshell. Therefore, the content of copper in the preserved eggshell was higher the duck eggshell.

4. Conclusions

From our study, we used a novel method - pressure technology - to pickle pidan. There were many pores on the eggshell of duck eggs. The surface of duck egg after acid pretreatment was rough. After pickling, some particles were observed around the pores of preserved eggshell, which reduced the size of the eggshell pores. The content of copper in the preserved eggshell was higher than duck eggshells.

5. Acknowledgements

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6. References

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