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# Establishment of a novel pork kidney lavage method and detection of heavy metals and antibiotics

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**Abstract**

The effectiveness and feasibility of a pork kidney lavage method to remove heavy metals and antibiotics from fresh pork kidney were investigated. This work provides a basis for the research and development of contaminant-free pork kidney foods. A comparative study was performed on pork kidneys with traditional treatment versus lavage treatment. The contents of Cu, Pb and Hg, 18 sulfonamides, 15 quinolones, ampicillin and cefalexin were detected by FAAS, GF-AAS, AFS and LC-MS/MS. The detection results of heavy metals and antibiotics showed that the content of Cu in pork kidney after lavage was significantly reduced, and antibiotics such as sulfachlorpyridazine and enrofloxacin were not detected. Therefore, lavage is an effective method to remove toxins from fresh pork kidney and can remove heavy metals and antibiotics to the maximum extent, providing a basic guarantee for the production of contaminant-free pork kidney foods.

**Keywords:** pork kidney; lavage; heavy metals; antibiotics; contaminant-free.

**Practical Application:** This lavage method is used to remove toxins from fresh pork kidney.

## 1 Introduction

Pork kidney, a nutritional food for humans, is rich in protein and essential trace elements. Pork kidney can be used to treat kidney deficiency, backache, spermatorrhea and so on. However, due to the simple and nonstandard treatment of commercially available fresh pork kidney, it retains a variety of substances harmful to the human body, such as various waste products and metabolites produced by metabolism, including uric acid and nitrogen- containing substances (Fathallah-Shaykh & Cramer, 2014); harmful substances ingested during feeding, mainly including heavy metals (Cu, Pb, Hg, etc.) and hormonal substances (Pei et al., 2020; Tuyet-Hanh et al., 2017); antibiotics that are ingested in various ways; and necrotic and decomposed substances produced during slaughter and preservation. In order to meet the increasing standard of living, the safety and taste of various pork products including pork kidney have been put forward higher requirements, so new solutions need to be found to meet this demand (Araújo et al., 2022; Huy et al., 2022).

In animal husbandry, some heavy metals are widely used in feed additives to improve the production performance of live pork and feed efficiency and to further stimulate and promote the rapid development of the live pork industry (Gao et al., 2020; Wang et al., 2018; Zhang et al., 2012). In addition, antibiotics, another important additive, are widely used in the treatment of animal diseases and as feed additives. Antibiotics play the role of preventing and treating diseases and promoting the growth of poultry and other agriculturally relevant animals (Angelakis, 2017; Dyar et al., 2020; Zhang et al., 2015). The kidney, as an important detoxification and metabolic organ of pork, commonly exhibits the accumulation of heavy metals and antibiotics (Clark & Parikh, 2020; Hosohata, 2016; Kieffer et al., 2016; Millet-Boureima et al., 2018; Torell et al., 2015). The traditional treatment of pork kidney is to cut the pork kidney longitudinally, separate the mucosa of the renal pelvis from the kidney parenchyma, and wash it under clean running water for 10 min. The problem with such a simple treatment is that a large number of harmful substances are still left in the pork kidney parenchyma and are then ingested and accumulated in the human body, leading to diseases (Fu & Xi, 2020; Pei et al., 2020).

Kidney lavage in vitro is one of the important steps of kidney transplantation (Jochmans et al., 2017; Tingle et al., 2019). However, it has not been reported whether kidney lavage can be used to remove toxins from kidneys. In this study, kidney lavage technology was used to remove toxins from fresh pork kidney, and the effects of lavage on the contents of heavy metals and antibiotics in pork kidney were evaluated by flame atomic absorption spectrometry (FAAS) (Schiller et al., 2019; Pohl et al., 2020), graphite furnace atomic absorption spectrometry (GF-AAS) (Habibollahi et al., 2019; Mimura et al., 2016), atomic fluorescence spectrometry (AFS) (Jin et al., 2020; Tao et al., 2020) and liquid chromategraphy tandem mass spectrometry(LC-MS/MS) (Chen & Fang, 2011; Duelge et al., 2017; Moreno-Bondi et al., 2009). The purpose of this study is to provide a scientific basis for the development of contaminant-free pork kidney foods.

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## 2 Materials and methods

### *2.1 Fresh pork kidney samples*

Kidneys were removed as soon as possible from the slaughtered Licha black pork, and the arteriovenous system and the proximal ureter were strictly protected to ensure that the length of the arteriovenous system and the proximal ureter were not less than 2 cm and 5 cm, respectively. The fat sacs on the kidney surface were quickly removed, and oil and blood clots on the kidney surface were cleaned with sterilized normal saline. Next, the kidney pelvis was repeatedly rinsed with normal saline injected through the proximal ureter, and the urine components and foreign proteins in the kidney collecting system were rinsed until clean.

### *2.2 Self-made flow-controllable lavage equipment*

The lavage equipment comprised an equipment body, a lavage device arranged inside the equipment body, an input pipeline connected with the lavage device, and a lavage fluid storage tank connected with the input pipeline. The input pipeline comprised a metering pump and an input pipe; the inlet of the metering pump was connected to the lavage fluid storage tank through a pipe, the input pipe was connected to the outlet of the metering pump and a pluralization of lavage pipes, and the lavage pipes were used to connect the pork kidney. The lavage device comprised a support frame, fixing frame and fixing groove. The support frame was arranged inside the device body, and a plurality of fixing frames were arranged on the support frame at intervals. A solenoid valve was arranged on the lavage pipe, a recovery pipe was arranged below the irrigation device, and a recovery pipe was connected to the end of the input pipe and the lavage fluid storage box.

### *2.3 Lavage of pork kidney*

Pretreated pork kidney samples were immersed in a fixed tank with normal saline at 4 °C, and the end of the lavage tube was inserted into the kidney artery and fixed firmly, with an insertion length of no less than 2 cm. At the same time, a negative pressure collection pipeline was inserted into the kidney vein. The processing temperature was kept as low as possible to prevent spoilage of the kidney, and the treatment procedure was compact to prevent coagulation of the kidney residual blood from affecting subsequent lavage. The lavage tube was inserted into the renal artery and vein at a depth of no less than 2 cm to ensure an accurate flow rate and no overflow of lavage fluid during the lavage process. The pressure and flow rate of the input pipeline were set at 60 mmHg and 50 mL/s, respectively, by the metering pump, and the lavage was continued under pressure for 5 min.

### *2.4 Traditional treatment of pork kidney*

The pretreated pork kidney samples were dissected longitudinally, the mucosa of the kidney pelvis was separated from the kidney parenchyma, and rinsed under clean running water for 10 min.

### *2.5 Detection of heavy metals*

Heavy metal contents of unlavaged and lavaged pork kidney samples were detected by BiWeiXinNuo (Shandong) Testing Technology Co., Ltd. Cu was determined by FAAS, and the detection was carried out according to the national standard of foodstuffs safety-determination of copper in foodstuffs (GB 5009.13-2017). Pb was determined by GF-AAS, and the detection was carried out according to the national standard of foodstuffs safety-determination of lead in foodstuffs (GB 5009.12-2017). Hg was determined by AFS, and the detection was carried out according to the national standard of foodstuff safety determination of total mercury and organic mercury in foodstuffs (GB 5009.17-2014).

### *2.6 Detection of antibiotics*

The antibiotic contents of unlavaged and lavaged pork kidney samples were detected by BiWeiXinNuo (Shandong) Testing Technology Co., Ltd, using LC-MS/MS. The detection of sulfonamides was carried out according to the determination of residues of sulfonamides in foodstuffs of animal origin-LC-MS/MS (GB/T 21316-2007). The detection of quinolones was carried out according to the determination of multi-residues of quinolones in food of animal origin for imports and exports-part 2: LC-MS/MS method (SN/T 1751.2-2007), analysis of fourteen quinolones in food of animal origin by high performance liquid chromatography tandem mass spectrometry (GB/T21312-2007), and the method for the determination of quinolones in animal tissues-LC-MS/MS method (GB/T 20366-2006). Ampicillin was detected according to the determination of penicillins residues in foodstuffs of animal origin-LC-MS/MS method (GB/T 21315-2007). Cefalexin was detected according to the determination of cefalexin, cephapirin and cefazolin residues in foodstuffs of animal origin for import and export-LC-MS/MS method (SN/T 1988-2007).

## 3 Results

### *3.1 Design of lavage equipment*

The pork kidney lavage equipment designed in this study is shown in Figure 1. The equipment mainly included the equipment body (1), support frame (2), input pipeline (3), fixed tank (4), pressure gauge (6), metering pump (7), lavage fluid storage tank (9), circuit control board (10), and base (11). The device body (1) and lavage fluid storage tank (9) were arranged on the base (11), and the roller (12) was mounted on the base (11). The metering pump (7) was installed above the lavage fluid storage tank (9). The input tube (3) was connected to the outlet of the metering pump (7), and the pressure gauge (6) was arranged on the input tube (3). The top of the lavage fluid storage tank (9) included a filling port (8). A support frame (2) was arranged inside the device body (1), many plural fixing frames (13) were separated on the left and right sides of the supporting frame (2), and the ends of the fixing frames (13) were connected with the fixing groove (4). The input tube (3) was provided with two branches that were fixed on the support frame (2), and a number of lavage tubes (14) were separately connected at intervals on each branch. The lavage tube (14) was attached to or placed on the mounting

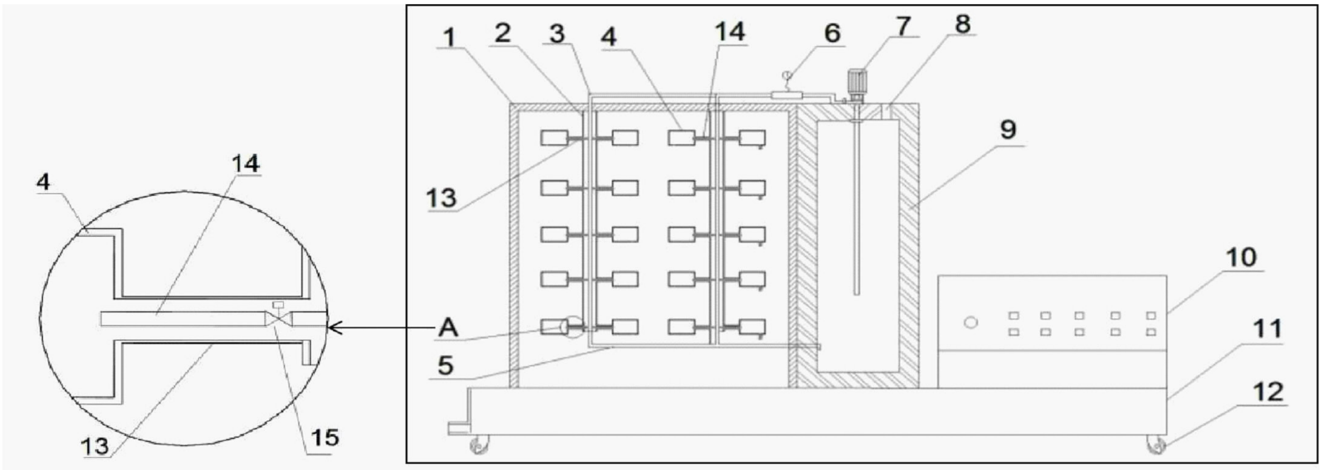


**Figure 1**. Structure diagram of kidney lavage equipment.

frame (13). A solenoid valve (15) was connected to the lavage pipe (14). The solenoid valve (15) was connected to the circuit control board (10). The end of the input pipe (3) branch was connected to the recovery pipeline (5) through a valve, and the recovery pipeline (5) was connected to the lavage fluid storage tank (9). After lavage, the recovery pipeline (5) recycled the excess lavage fluid to the lavage fluid storage tank (9). Each lavage tube corresponded to a fixed slot (4), which was used to place the animal kidney. The opening and closing of the solenoid valve (15) on each lavage pipe was controlled by a circuit control board (10) and further controlled the connection between the douche tube (14) and the input tube branch. The support frame (2) and fixing frame (13) were constructed of welded steel pipes. Because the equipment is simple to operate and has a definite lavage effect, it can be used for large-scale production and has great economic value and market value. This study provides a preliminary basis for the further processing of animal kidneys and the development of high value-added products.

### *3.2 Color difference in pork kidney samples before and after lavage*

Compared with unlavaged pork kidney, the lavaged pork kidney was grayish white, and their colors were significantly different (Figure 2).

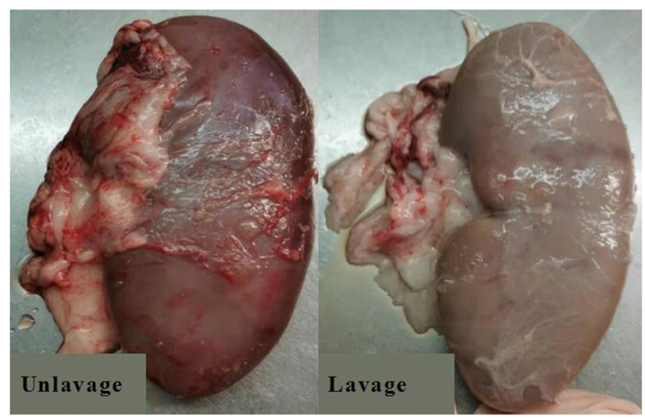


**Figure 2**. Color contrast before and after lavage.

### *3.3 Detection of heavy metals*

Heavy metal contents were detected in unlavaged and lavaged pork kidneys, and Pb and Hg were not detected in any samples. However, the content of Cu decreased from 5.8 mg/kg to 4.4 mg/kg after lavage (Table 1). The results showed that heavy metals could be removed after lavage.

### *3.4 Detection of antibiotics*

The levels of antibiotics in unlavaged and lavaged pork kidneys were detected, including 18 sulfonamides (Table 2), 15 quinolones (Table 3), ampicillin and cefalexin (Table 4). Among them, 33 antibiotics were not detected in either unlavaged or lavaged kidneys. However, the contents of sulfachlorpyridazine and enrofloxacin in unlavaged kidneys were 57.8 μg/kg and 11.8 μg/kg, respectively, and neither of these two antibiotics was detected in the lavaged kidney.

## 4 Discussion

Pork kidney serves as a nutritional food for humans. The traditional kidney treatment process is to cut the pork kidney longitudinally, separate the mucous membrane of the kidney pelvis from the kidney parenchyma, and wash it under clean running water. Therefore, a large amount of harmful substances remain in the kidney parenchyma due to simple handling and are then ingested, causing diseases (Pei et al., 2020; Wu et al., 2016).

To solve this problem, we designed lavage equipment for cleaning fresh animal kidneys. This treatment method could remove contaminants and other residual substances in blood vessels inside the kidney, improving its safety for consumption. In today's global economy, food safety is receiving increasing

**Table 1**. Contents of heavy metals before and after lavage.

| Test item | Unit | Test basis | Value (Unlavage) | Value (Lavage) |
|---|---|---|---|---|
| Pb | mg/kg | GB5009.12-2017 | Non-Detected (< 0.04) | Non-Detected (< 0.04) |
| Cu | μg/kg | GB5009.13-2017 | 5.8 | 4.4 |
| Hg | μg/kg | GB5009.17-2014 | Non-Detected (< 0.01) | Non-Detected (< 0.01) |

**Table 2**. Contents of sulfonamides before and after lavage.

| Test item | Unit | Test basis | Value (Unlavage) | Value (Lavage) |
|---|---|---|---|---|
| Sulphaguanidine | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfaphenazole | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfabenzamide | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfapyridine | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfacetamide | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfadimethoxine | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfametoxydiazine | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfadoxine | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfadimidine | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfamethoxazole | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfamethazine | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfamethoxypyridazine | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfamonomethoxine | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfaquinoxaline | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfachlorpyridazine | μg/kg | GB/T 21316-2007 | 57.8 | Non-Detected (< 50) |
| Sulfadiazine | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfathiazole | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |
| Sulfanitran | μg/kg | GB/T 21316-2007 | Non-Detected (< 50) | Non-Detected (< 50) |

**Table 3**. Contents of quinolones before and after lavage.

| Test item | Unit | Test basis | Value (Unlavage) | Value (Lavage) |
|---|---|---|---|---|
| Orbifloxacin | μg/kg | SN/T 1751.2-2007 | Non-Detected (< 10.0) | Non-Detected (< 10.0) |
| Marbofloxacin | μg/kg | SN/T 1751.2-2007 | Non-Detected (< 10.0) | Non-Detected (< 10.0) |
| Difloxacin | μg/kg | SN/T 1751.2-2007 | Non-Detected (< 10.0) | Non-Detected (< 10.0) |
| Sparfloxacin | μg/kg | SN/T 1751.2-2007 | Non-Detected (< 10.0) | Non-Detected (< 10.0) |
| Fleroxacin | μg/kg | GB/T 20366-2006 | Non-Detected (< 1.0) | Non-Detected (< 1.0) |
| Danofloxacin | μg/kg | GB/T 21312-2007 | Non-Detected (< 3.0) | Non-Detected (< 3.0) |
| Enrofloxacin | μg/kg | GB/T 21312-2007 | 11.8 | Non-Detected (< 3.0) |
| Ciprofloxacin | μg/kg | GB/T 21312-2007 | Non-Detected (< 8) | Non-Detected (< 8) |
| Lomefloxacin | μg/kg | GB/T 21312-2007 | Non-Detected (< 3.0) | Non-Detected (< 3.0) |
| Norfloxacin | μg/kg | GB/T 21312-2007 | Non-Detected (< 6) | Non-Detected (< 6) |
| Pefloxacin | μg/kg | GB/T 21312-2007 | Non-Detected (< 6) | Non-Detected (< 6) |
| Sarafloxacin | μg/kg | GB/T 21312-2007 | Non-Detected (< 6) | Non-Detected (< 6) |
| Enoxacin | μg/kg | GB/T 21312-2007 | Non-Detected (< 10) | Non-Detected (< 10) |
| Ofloxacin | μg/kg | GB/T 21312-2007 | Non-Detected (< 3.0) | Non-Detected (< 3.0) |
| Cinoxacin | μg/kg | GB/T 21312-2007 | Non-Detected (< 6) | Non-Detected (< 6) |

**Table 4**. Contents of ampicillin and cefalexin before and after lavage.

| Test item | Unit | Test basis | Value (Unlavage) | Value (Lavage) |
|---|---|---|---|---|
| Ampicillin | μg/kg | GB/T 21315-2007 | Non-Detected (< 5) | Non-Detected (< 5) |
| Cefalexin | μg/kg | SN/T 1988-2007 | Non-Detected (< 2) | Non-Detected (< 2) |

attention worldwide, the basis of which is to provide methods to ensure food quality and awareness of safety knowledge so as to avoid foodborne diseases (Hossen et al., 2021; Huy et al., 2022).

The kidney is divided into the kidney parenchyma and kidney pelvis, and the kidney parenchyma is divided into the outer cortex and inner medulla. The renal cortex is mainly composed of renal tubules and glomeruli. The glomerular filtrate is filtered through the capillary walls of the glomerulus, which are made up of endothelial cells, the basement membrane of the glomerulus, and epithelial cells. This equipment makes full use of the structural features of the nephron and the lavage pressure, flow rate, and time were set to 60 mmHg, 50 mL/s, and 5 min, respectively. Blood in the glomerular capillaries and other harmful substances were flushed out of the kidney through kidney veins by lavage fluid, the lavage flow rate of the kidney artery was close to the normal physiological value during lavage, and the integrity of the glomerular filtration membrane was protected. At the same time, isotonic normal saline could also pass through the glomerular filtration membrane, and the harmful substances such as antibiotics, heavy metals and hormones remaining in the kidney medulla could be flushed into the collection system and discharged from the pork kidney through the proximal ureter. From the economic point of view, this equipment is simple to operate and can process multiple pork kidneys at the same time, high yield and high efficiency, is especially suitable for mass production in the market and provides an equipment platform for the further processing of pork kidneys. In addition, from the perspective of environment, the lavaged pork kidney further ensures food safety, is more easily accepted by people, and further reduces the environmental pollution caused by waste.

Three heavy metals and 35 antibiotics were detected in unlavaged and lavaged pork kidneys. The Cu content decreased from 5.8 mg/kg to 4.4 mg/kg after lavage, and the antibiotic contents of sulfachlorpyridazine and enrofloxacin decreased from 57.8 μg/kg and 11.8 μg/kg, respectively, to undetected levels. All results strongly demonstrate the effectiveness of the lavage equipment in removing heavy metals and antibiotics. This study is a preliminary experimental study on toxin removal from pork kidneys, which provides a scientific basis for the development of contaminant-free pork kidney foods.

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