Removal of Streptomycin from Honey by Cation-exchange Resin

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ABSTRACT: The presence of streptomycin (STR) in honey has potentially undesirable effects on humans. This study evaluated the effect of conventional processing on STR levels and investigated a new approach to further removing STR using various types of resins. The use of cation-exchange resin (LS-904) after conventional processing, significantly reduced STR residues, with loss rate of approximately 100%. The optimal adsorption time and temperature were 60 min and 45°C, respectively. Moreover, compared with an anion-exchange resin (LS-905) or macroporous-adsorption resins (LS-200, NKA-9), LS-904 was more effective in removing STR from honey. The processed honey can be widely used as natural sweeteners.

INTRODUCTION

An antibiotic is a type of pharmaceutical that has the ability to kill or inhibit the growth of microorganisms. Antibiotics are extensively used in agriculture. In the field of apiculture, bees are treated with antibiotics to fight diseases such as American and European foulbrood diseases [1]. In the European Union, it is illegal to treat honeybees with antibiotics. However, in some developing countries, antibiotics are still used for this purpose; therefore, antibiotic-contaminated honey products can be found in the global marketplace [2].

Streptomycin (STR) is an aminoglycoside antibiotic which is produced by Streptomyces griseus. It can effectively inhibit gram-positive and gram-negative bacteria by causing codon misreading, which thus inhibits protein synthesis and leads to the death of the microbial cells [3]. Due to its inhibitory effects, STR has numerous applications in a wide range of human therapies and animal husbandry and agricultural practices, including apiculture (keeping honeybees) [4]. However, when this antibiotic is overused, STR residues may appear in many foodstuffs, e.g., meat products, animal livers, milk and, above all, in honey. Although it is debatable whether STR residues have a direct impact on human health, many cases of allergic attacks have occurred in recent years, and STR can induce severe skin rashes [3,5]. Furthermore, certain other negative effects of this antibiotic, such as increasing the risk of hearing loss and toxicity to the kidneys, have also been claimed [6]. The long-term use of STR can induce bacterial resistance, and some studies have shown that E. coli, Salmonella and Shigella may carry this resistance [7].

Therefore, to protect human health against the dangers of STR residues, maximum residue limits (MRLs) for some food products were established by certain organizations. For example, the European Commission stipulated that the MRLs for STR in milk, porcine kidney and porcine muscle are 200, 1,000 and 500 mg kg⁻¹, respectively [7]. Moreover, Switzerland and Germany imposed a regulation that the MRL in honey is 20 µg kg⁻¹. Due to concerns for human health, many countries have banned the use of STR in natural products such as honey [7–8].

Honey is a sweet substance, produced from the nectar of flowers by honeybees. From ancient times, honey has been favored for its nutritional and medicinal qualities such as antibacterial and dermatological disorders [9–10]. Moreover, the antioxidant activity of honey can reduce the risk of degenerative diseases of aging [11]. Unfortunately, honey is vulnerable to contamination with antibiotics, such as STR, which are applied to treat honeybee diseases. Generally, once the amount of STR residues reaches the MRL, the tainted honey should be discarded. However, this practice may result in great waste because tainted honey still contains valuable substances that are underutilized. Considering these reasons, controlling the level of STR residues in honey is extremely urgent.

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It is gratifying that several studies have shown that the processing of honeys is highly effective in reducing the detrimental effects of organophosphorus compounds and antibiotics [12–14]. However, few studies have investigated the effect of honey processing on the level of STR residues in honey. And there is little research on removing STR from honey. The present study aimed to determine how the honey processing steps, such as preheating, filtration, vacuum concentration, and pasteurization, affect the level of STR residues and to explore a simple, highly efficient, economical and safe method to remove STR from honey.

MATERIALS AND METHODS

Chemicals

Analytical grade STR and phosphoric acid were obtained from Sigma-Aldrich (St. Louis, MO, USA).

An STR quantification kit (batch number HE09024) was provided by Huaan Magnech Co., Ltd. (Beijing, China) and was used to conduct the enzyme-linked immunosorbent assay (ELISA), which utilized a specific rabbit anti-streptomycin antibody. The STR kit included all of the solvents and reagents necessary for the ELISA.

Adsorbents

The following resins were prepared for the study: the cation-exchange resin LS-904, the anion-exchange resin LS-905, and the macroporous adsorption resins LS-200 and NKA-9. The LS-904, LS-905 and LS-200 resins were provided by Xi'an Lansen Exchange and Adsorbent Material, Ltd. (Xi’an, China), and the NKA-9 resin was purchased from Tianjin Nanda Adsorbent Material, Ltd. (Tianjin, China).

Honey Samples

Preparation of Raw Honey Samples

The raw honey samples were provided by beekeepers and were confirmed to be antibiotic-free.

Preparation of Spiked Honey Samples

The spiked honey samples were prepared according to the following steps. First, defined amounts of the STR standard were introduced into the raw honey samples (200 g); next, the mixtures were homogenized in a water bath for 4 h at 25°C; the samples were then stored in the refrigerator until use.

ELISA Procedure

The concentration of STR in the honey samples was determined with an ELISA according to the manufacturer’s instructions. The procedure was as follows: the spiked honey sample (2 g) and purified water (2 mL) were mixed to prepare the diluted honey sample. Then, phosphoric acid (0.04 mol L⁻¹, 4 mL) was added to the diluted honey sample, and the mixture was thoroughly homogenized. The pH value of the sample was adjusted to approximately 8.0 using sodium hydroxide (1 mol L⁻¹, 350 µL). After that, the STR-containing diluent was prepared by mixing the concentrated STR sample diluent and purified water at a ratio of 1:1 (v:v). The homogenates were centrifuged at 4,800 rotations/min (rpm) for 10 min, and the supernatants (100 µL) were removed and added to the STR-containing diluent, the samples were thoroughly mixed.

The absorbance of a 50 µL aliquot of the aqueous layer at 450 nm was measured with an ELISA Reader (Infinite M200 Pro, Tecan Austria GmbH, Grödig, Austria). The STR levels were calculated using a calibration curve. The concentration of STR was expressed in the form of µg kg⁻¹ honey. In this study, the limit of quantification of STR was 4 µg kg⁻¹. The average recovery rates ranged from 95.6–97.3% for all of the samples.

Processing of the Spiked Honey Sample

The procedure for processing the honey was organized into 4 consecutive steps according to the conventional method [15], including preheating, filtration, vacuum concentration, and pasteurization. After each step, the samples were collected, and the concentration of STR was determined using the ELISA.

Static Adsorption Experiments

Pretreatment of the Resins

Four different resins, i.e., the LS-904, LS-905, LS-200, and NKA-9 resins, were selected for the study, which required different pretreatments before use.

The cation-exchange resin (LS-904) and the anion-exchange resin (LS-905) were soaked in HCl (4%) and
NaOH (4%), respectively, for 2 h using a volume of the corresponding solvent that was three times greater than that of the resin volume. The macroporous adsorption resins (LS-200, NKA-9) were first soaked in ethanol (95%) for 24 h and then rinsed with purified water until the resins were ethanol-free; finally, these resins were soaked in a mixture of HCl (5%) and NaOH (5%) at a ratio of 1:1 to eliminate porogenic agents and monomers that had been trapped within the pores during the synthesis process. Eventually, all of the resins were rinsed using purified water until the resins were chemically neutral.

**Screening the Adsorption Resins**

To determine the optimal resin among the LS-904, LS-905, LS-200 and NKA-9 resins, the following experiment was conducted. Pre-weighed hydrated resins were added to a diluted honey sample (25 g) [honey:purified water (m:m) at 1:1.5] containing a certain concentration of STR, and the mixtures were stirred in a water-bath at the rate of 120 rpm at 25°C for 1 h. Then, the percentage of STR remaining in the honey was determined using the ELISA.

**STR-adsorption Experiment**

In this experiment, the influence of resin dose, contact time and temperature on adsorption process were explored. The diluted honey sample containing a certain concentration of STR (25 g) was added to the selected resin and swirled in a water-bath shaker at 120 rpm at 25°C. The amount of the resin was increased from 0.5–3.0 g. After 90 min, 4 g of honey sample was removed to determine the STR content. To study the effect of time, the adsorption time was increased from 30–90 min (in increments of 30 min), while the water-bath temperature and amount of resin were maintained at 25°C and 2 g, respectively. Similar methods were applied to assess the effect of temperature on STR adsorption by increasing the temperature from 25–65°C (in increments of 10°C).

**Statistical Analysis**

The assays were performed in triplicate, and the results were expressed as the mean values with the standard deviation (SD). The differences between values with \( P < 0.05 \) were considered significant. The statistical analyses were performed using Origin software, version 8.0 and Microsoft Office Excel 2010.

**RESULTS AND DISCUSSION**

**Effect of the Processing Procedures on the Streptomycin Level**

The effect of the processing procedures on the reduction of the STR level was determined by performing an ELISA, and the results are presented in Figure 1. The spiked honey samples were preheated at 45°C for 60 min to make them liquid. After the preheating processing, the STR level was decreased by 34.84%, indicating that STR is a thermally unstable antibiotic. A similar result was obtained in a previous study by Landerkin and Katznelson [16].

The preheated honey samples were then filtered to remove the suspended particles, including the bodies of honeybees, beeswax particles, and pollens. The decrease of STR content that occurred during the filtration process was not significant. Nevertheless, it has been shown that filtration significantly contributes to removing other types of antibiotics and organophosphorus insecticides from honey. This occurrence appears to depend on the degree of lipophilicity of the antibiotics (e.g., chloramphenicol) and parathion, which favors the association of these compounds with the fatty fractions [14]. However, STR does not tend to be retained in pollen or beeswax due to the hydrophilicity of STR. Therefore, the STR concentration had nearly no reduction after the filtration processing, which is consistent with the results of a study by Chen et al. [17].

Next, vacuum preconcentration was conducted at 55°C and 0.08 MPa for 45 min. Vacuum preconcentration plays a vital role in eliminating microorganisms and reducing the moisture content to a degree that retards fermentation [14]. In addition, vacuum precon-

![Figure 1. Changes of the streptomycin (STR) content in honey during conventional processing. Different lower case letters indicate significant differences at \( P < 0.05 \).](image-url)
concentration contributes to maintaining the honey in a liquid state for a long period due to melting the invisible crystals in honey [15]. After this procedure was performed, the STR residue was decreased by 54.9%.

For honey to become a commodity, it must be pasteurized at 85°C for 15 min. The mean loss of STR caused by pasteurization process was 28.54%, indicating that STR is unstable at high temperatures.

**Screening to Determine the Optimal Resin**

Despite being effective, the normal processing procedures did not completely remove the STR from honey. The level of STR residues after the 4 procedures were performed was 651 µg kg$^{-1}$, which far exceeded the MRL. Therefore, using absorption resins may be an efficient method to remove STR from honey. In the static adsorption tests, the performance of the 4 resins in removing STR was determined, as shown in Figure 2. The adsorption rates of the LS-905, NKA-9, LS-904, and LS-200 resins were 26.76%, 21.29%, 65.77%, 24.09%, respectively. Obviously, the LS-904 resin had the highest adsorption rate.

Compared with the macroporous adsorption resins (LS-200, NKA-9), the performance of ion-exchange resin is related to the properties of the exchange groups. Ion-exchange resins generally carry cations or anions that are exchanged with similarly charged ions in solution via electrostatic interactions. Hence, the ionic groups in a solution migrate to the resins and the other ion groups migrate in the opposite direction until electroneutrality is achieved. The LS-904 resin is a cation-exchange resin, whereas the LS-905 resin is an anion-exchange resin. The highest adsorption rate caused by the LS-904 resin could be attributed to the fact that the STR became $\text{Str-H}_3^{3+}$ in the honey sample, which had a pH of 3.2–4.5, so that the $\text{H}^+$ ions produced by the LS-904 resin would be exchanged with $\text{Str-H}_3^{3+}$. The schematic diagram of this process is illustrated in Figure 3. Therefore, considering its adsorption capacity, the LS-904 resin was selected as the optimal resin to remove STR from honey.

**Effect of the Adsorption by the Cation-exchange Resin on the STR Levels**

In the static adsorption assay, the amount of LS-904 resin, the adsorption time and the temperature were varied to determine the optimal conditions. The results were depicted in Figure 4.

As shown in Figure 4(a), the optimal amount of LS-904 resin was determined. The STR content in honey samples decreased with an increase in the amount of resin and finally attained an equilibrium above 80 g LS-904 resin $\text{kg}^{-1}$ honey, at which the STR residue was nearly zero. The efficiency of STR-residue removal using this method was satisfactory, reducing the STR content from 76.8 µg kg$^{-1}$ to nearly 0. The final STR content was far below the MRL, which indicated that the LS-904 resin is highly suitable for removing STR.

The effect of time on the adsorption of STR by the LS-904 resin was observed, and the results are shown in Figure 4(b). The STR level, which was initially at 68 µg kg$^{-1}$, decreased sharply within the first 30 min, then stabilized between 30 and 60 min. The STR level eventually remained unchanged after approximately 60 min, demonstrating that the maximum adsorption efficiency had been attained. More significantly, the reduction rate was approximately 100%. Based on these results, the optimum adsorption period is 60 min.

Figure 4(c) illustrates the effect of temperature on the adsorption of STR residues in honey by the LS-
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When the temperature was increased from 25–45°C, the rate of STR adsorption increased to 86.72%. It is noteworthy that the adsorption rate was apparently greatest at 45°C, at which the STR content was approximately 10 µg kg⁻¹, lower than the MRL. However, after this point, the adsorption rate began to decrease with increasing temperature. Moreover, an undesirable adsorption process is likely to impair the quality of the honey. As reported by Turkmen et al. [18], both high-temperature and a long exposure directly affect the bioactivities and qualities of honey, particularly some of the antioxidant properties. Therefore, although the STR content in honey was reduced by increasing the temperature, taking all the facts into consideration, 45°C was chosen as the most appropriate adsorption temperature.

CONCLUSIONS

This study investigated the effect of conventional honey processing on the removal of STR residues from honey and explored an efficient method of further reducing the STR concentration using ion-exchange resins. The results indicated that vacuum concentration was the most efficient process for removing STR residues from honey. In addition, among the four candidate resins (LS-904, LS-905, LS-200 and NKA-9), the LS-904 resin had the highest adsorptive capacity for STR in honey, and the best adsorption time and temperature were determined to be 60 min and 45°C, respectively. By leveraging the method proposed in this paper, tainted honey could be widely used in food industry as a safe and acceptable natural sweetener.

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