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Analytical Methods

# Determination of purine contents in different parts of pork and beef by high performance liquid chromatography

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ABSTRACT

Determination of adenine, hypoxanthine, guanine and xanthine in different parts of pork and beef using high performance liquid chromatography was described. Chromatographic separation was carried out on Waters Atlantis $T_3$ column (4.6 mm × 250 mm × 5 μm) with column temperature at 30 °C. The mobile phase contained 99% 10.0 mmol/L ammonium formate solution at pH 3.6 and 1.0% methanol. Chromatography was achieved at a flow rate of 1.0 mL/min and detection wavelength at 254 nm. The results indicated that total purine amounts in pork rump and beef sirloin were higher than those in other parts ($P < 0.05$). The principal purine bases were hypoxanthine and adenine, and hypoxanthine content was the most highest in all samples ($P < 0.05$). As pork rump and beef sirloin contain considerable amounts of total purine and uricogenic purine base, we suggest that excess consumption of them be avoid, whereas pork loin chop and beef rib eye are more suitable for a low-purine diet.



## 1. Introduction

Uric acid is the end product of purine metabolism in human body (Johnson, Titte, Cade, Rideout, & Oliver, 2005; Merriman & Dalbeth, 2011). Longstanding elevated serum uric acid levels can lead to hyperuricemia or gout, which is a highly prevalent and excruciatingly painful inflammatory arthritis caused by accumulation of monosodium urate crystals (MSU) within joints and other soft tissues (Smith, Díaz-Torné, Perez-Ruiz, & March, 2010). Typically, the risk for gout tends to be higher among men than women in all age groups, although there is an equalization of the sex ratio with advancing age (Smith, Bracken, & Smith, 2011). The incidence and prevalence of hyperuricemia and gout appear to be increasing worldwide in recent years (Ciancio, Bortoluzzi, & Govoni, 2012; Zhu, Pandya, & Choi, 2011). In addition to severe pain and suffering, previous study has also reported that the overall disease burden of hyperuricemia and gout remains substantial and is growing (Choi, Gao, & Curhan, 2009).

Dietary purine intake has an important influence on serum uric acid levels (Yamaoka et al., 2010; Kedar & Simkin, 2012). Several studies have also confirmed that low-purine diet is very important to patients with hyperuricemia and gout (Choi, Atkinson, Karlson, Willett, & Curhan, 2004; Shmerling, 2012; Suresh & Das, 2012).

Pork and beef are one of the most widely consumed animal foods in the world, but previous studies have described that they contain large amounts of purine (Brulé, Sarwar, & Savoiet, 1988; Doghramji & Wortmann, 2012). Though several studies were done concerning determination of purine content in pork or beef (Clariana, Gratacós-Cubarsí, Hortós, García-Regueiro, & Castellari, 2010; Yang, Zhang, Liu, & Zhang, 2012), no information, to our knowledge, about purine contents in different parts of pork and beef has been reported. Pork and beef are divided into different parts (pork rump, pork tenderloin, pork fore hock, pork loin chop, beef sirloin, beef rib finger, beef blade, beef rib eye) to consume after an animal is slaughtered, so the new perspective may be more helpful for the prevention and management of hyperuricemia and gout for patients and physicians.

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Several methods for quantifying purine amount in food have been described. Among these methods, high performance liquid chromatography (HPLC) is still predominant (Fukuuchi et al., 2013; Trugo, MacRae, & Dick, 1983), simple, attainable and widely used technique (Blanchard, Weber, & Shearer, 1990; Clariana et al., 2010; Cox, Loscombe, & Upfield, 1976; Fukuuchi et al., 2013; Warthesen, Waletzko, & Busta, 1980). Nevertheless, only several HPLC methods are available about determination of purine in pork and beef (Yang et al., 2012) and there is no information on purine amounts in different parts of pork and beef are analysed using HPLC.

The objective of the present study was to simultaneously quantify the levels of adenine, guanine, hypoxanthine and xanthine in different parts of pork and beef by a new developed HPLC.

## 2. Materials and methods

### 2.1. Standards and chemicals

Standards were obtained from Sigma Chemical Company (St. Louis, MO, USA). The purines used in this study were adenine, guanine, hypoxanthine and xanthine, which were chromatography-grade and all assayed at more than 98% purity. Ammonium formate, perchloric acid and formic acid were purchased from Tianjin Guangfu Institute of Chemical Engineering Co, Ltd (Tianjin, China), they were all guaranteed reagent (GR). Methanols, acetonitrile, isopropanol HPLC-grade were obtained from DIMA Chemical Company.

### 2.2. Sample collection

Samples of pork rump ($n = 6$), pork tenderloin ($n = 6$), pork fore hock ($n = 6$), pork loin chop ($n = 6$), beef sirloin ($n = 6$), beef rib finger ($n = 6$), beef blade ($n = 6$) and beef rib eye ($n = 6$), were purchased from several local supermarkets. Different parts of pork and beef were identified by one of the authors (Shengzhong Rong). After that, each sample was homogenised with meat chopper, stored at −20 °C and analysed within 3 days.

### 2.3. Sample solution preparation

A sample (0.2000 g) was placed in a glass centrifugal tube (10.0 mL), and two millilitres of 6.0% concentration of perchloric acid was added and mixed by vortexing for 40 s. The mixture was then extracted for 60 min in boiling water bath and mixed for 40 s at 30, 40 and 50 min, respectively by vortexing. After the extract was chilled in ice, the pH of which was adjusted to 7.0 with concentration of 2.0 mol/L potassium hydroxide solutions and 5.0% formic acid. Extract pH was finally adjusted to 3.6 using 5.0% formic acid and was then diluted to 10.0 mL with 10.0 mmol/L ammonium formate solution at pH 3.6. Next, the sample solution was passed through a 2.5 mL disposable syringe filter containing a 0.22 μm Millipore filter. At last, the filtrate was then directly analysed.

### 2.4. Chromatographic conditions

Analysis was carried out with Waters Alliance 2695 liquid chromatography system (Waters, Milford, MA) equipped with a binary pump system (Waters, Milford, MA), a Waters 2487 UV detector (Waters, USA), an autosampler and heated column compartment. Separation was achieved through the usage of a Waters Atlantis $T_3$ chromatographic column (4.6 mm × 250.0 mm × 5.0 μm) (Waters, USA) with a Waters guard column (Waters, 2.1 × 10.0 mm). The temperature of column heaters was set and maintained at 30 °C and the sample compartment was maintained at 6 °C. A 10 μL sample solution during each injection was eluted with mobile phase consisted of 99% 10.0 mmol/L ammonium formate buffer solution at pH 3.6 and 1% methanol at a constant flow rate of 1.0 mL/min.

Data analysis was performed by applying Waters data analysis software (Waters, USA). HPLC-grade water (18 mΩ), which was used for the mobile phase and preparation of all solutions, was obtained by a Milli-Q system (Millipore, Milford, MA).

### 2.5. Method evaluation and quantification

The standard curves, created on each of the analysis day, were prepared over concentration ranges of 0.05–20.0 μg/mL. The intra-day and inter-day precisions were evaluated by analysing pork sample six times within a day and by analysing the pork sample in six successive days respectively. Accuracy of the method was tested by adding certain amounts of individual purine base standard to the pork sample (four types of purine base contents had been predetermined in our previous study). In each case a mixture of standards with 50%, 100% and 200% of the quantified levels of constituents was spiked into the sample, which was subjected to the extraction procedure of sample and then analysed by HPLC in triplicate. Compound quantification was achieved by regression analysis of compound peak area ($y$) against concentration ($x$).

### 2.6. Statistical analysis

Each sample was analysed in triplicate. Data was expressed as mean ± SD. Statistical analysis was performed with SPSS 16.0 software (version 16.0 for windows) and statistical significance was evaluated using analysis of variance (ANOVA). Results were considered significant at probability ($p$) values less than 0.05.

## 3. Results and discussion

### 3.1. Establishment of the HPLC method

In this study, several conditions were assayed in developing appropriate method, including mobile phase, column and column temperature, flow rate, wavelength and extraction procedure of purine.

Both potassium phosphate monobasic solution and ammonium formate solution, which were used as mobile phases, were tested to obtain a good chromatographic separation with standards solutions. The potassium phosphate monobasic solution range investigated was 20.0 mmol/L, 40.0 mmol/L, 60.0 mmol/L, 80.0 mmol/L and 100.0 mmol/L. However, four types of purine could not be separated efficiently. The ammonium formate solution and potassium phosphate monobasic solution/methanol mobile phase systems were attempted and then optimised. The ammonium formate solution ranges tested were 2.0 mmol/L, 5.0 mmol/L and 10.0 mmol/L. The results demonstrated that 99:1 10.0 mmol/L ammonium formate solution/methanol allowed the adequate resolution of adenine, guanine, hypoxanthine and xanthine, and the best resolution was achieved when the pH of ammonium formate was adjusted to pH 3.6 with formic acid. Therefore, 99:1 10.0 mmol/L ammonium formate solution at pH 3.6/methanol were used as the mobile phase.

To separate purine base in a short time, we subsequently evaluate different columns and column temperatures. Waters Atlantis $T_3$ column could yield good peak shapes and perfect selectivity, so Waters Atlantis $T_3$ column was chosen in the present study. With regard to column temperature, different temperatures were tested (20 °C, 25 °C and 30 °C), the best resolution was obtained when the temperature was set at 30 °C.