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# HPLC Analysis of Sinapine Thiocyanate in *Raphanus sativus* and Determination of Its Involvement in Gastric-Intestinal Function

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The sinapine thiocyanate from the fresh and cooked juice of *Raphanus sativus* was analyzed by a high-performance liquid chromatographic (HPLC) system equipped with a $C_{18}$ column and an UV detector. The column temperature was 25 ºC. The mobile phase was acetonitrile and 3 % acetic acid (10: 90) at a flow rate of 0.8 mL/min. The sinapine thiocyanate in fresh and cooked juice was 3.33 and 1.49 µg/g, respectively. It is notable that the cooked juice of radish promoted gastrointestinal transit, while the fresh juice exerted remarkable impact on gastric emptying. We thus reason that sinapine thiocyanate may be positively correlated with gastric emptying, yet has little impact on gastrointestinal transit. This study provides basis for both food processing and in-depth exploration of the roles of radish ingredients in ameliorating gastrointestinal transit.

**Keywords: HPLC, *Raphanus sativus*, Sinapine thiocyanate, Gastric emptying, Gastric-intestinal function.**

## INTRODUCTION

*Raphanus sativus* L., also known as radish, belongs to the genera cruciferae. As a favorite vegetable, its root has long been used for preparing Chinese cuisine. In addition, as a traditional medicine, it is applied to cure gastric dyspepsia fullness, hiccough and gastric disorder that causes nausea[1,2]. In fact, radish contains a wealth of biologically active components, including sinapine thiocyanate, methyl-mercaptan, β, γ-hexenol, α,β-methylbutanol, linoleic acid, eruci acid, polysaccharide, linolenic acid, alkaloid, phenol, phytosterols, flavonoid, *etc*. These components execute wide pharmacological actions[3,4].

Normally, people eat the fresh or cooked radish, but the reason behind has not been well documented. Specially, the intrinsic relationship between the active ingredients and gastrointestinalmotility remains unknown. The lack of basic research limits its clinical application in the treatment of gastrointestinal motility disorders. Towards development of a healthy food as well as medicine, it is imperative to unravel the roles of active ingredients in promoting gastrointestinal motility.

In this study, the crude extract was prepared and animal experiments were performed. HPLC analysis of the sinapine thiocyanatein fresh and cooked juice of radish was to provide a guideline for both routine cooking and medicine manufacturing. Animal experiments were to determine the effect of radish juice on gastric motility in mice and particularly to elucidate the pharmacological difference between the fresh and cooked juice.

## EXPERIMENTAL

The fresh roots of *R. sativus* were purchased from the supermarket in Beijing (China), divided into two groups, cooked or squeezed, respectively for fresh juice. The sinapine thiocyanate was purchased from China Biological Products Institute. Domperidone was product of Xi'an Yangsen Pharmaceutical Co., Ltd. Male and female (by half) ICR mice weighting an average of 20-25 g were provided by Vital River Laboratory Animal Technology Co. Ltd, Beijing China. Mice were housed in acrylic cages inside a sound proof chamber, under standardized conditions in a room maintained at a constant temperature (20-24 ºC) on a 12 h light/dark cycle. Mice had free access to water and food. The animal testing followed the guideline of the Ethical Committee on Animal Experimentation.

**Preparation of *R. sativus* extracts (or juice):** The fresh roots of *R. sativus* were thoroughly washed with distilled water, cut into strips and squeezed for the juice. The juice was immediately filtered through a 0.45 µm membrane and the sediment was discarded. The juice was concentrated by vacuum freezing, stored at -80 °C until use. 1 mL fresh extract was roughly equivalent to 15 g of *R. sativus* sample.

**Preparation of the cooked juice:** The fresh radish was eluted in distilled water with a mass to volume ratio of 1:30

(g/mL) at 90 °C, 0.5 h for three times. The extract was filtered through Whatmann No. 1 paper and subsequently concentrated by rotary evaporation instrument. As a result, the aqueous extract (cooked juice) was acquired, namely 1 mL extract is equivalent to 15 g of radish sample.

**Quantitative analysis of sinapine thiocyanate:** Juice was analyzed by reversed-phase HPLC: Shimadzu, LC-10AT vp HPLC pump, CTO-10AS vp thermostated column compartment, SPD-10A vp detector and controller. The juice was filtrated through 0.45 μm filtration membrane (Millipore, Bedford, MA). To optimize chromatogram conditions, we employed two different chromatogram columns:Platisil ODS and diamonsil $C_{18}$ (4.6 × 250 mm, 5 μm, DIKMA, American), which were tested at different wavelength (229, 330 and 333 nm) in combination with three mobile phase systems (water: methanol; water:acetonitrile: formic acid; acetonitrile: 3 % acetic acid). Moreover, different flow rate (0.6, 0.8 and 1 mL/min) and temperature (20, 25 and 30 ºC) were also alternated for this optimization. Consequently, the optimum HPLC conditions were listed as follows: Column:Diamonsil $C_{18}$ (250 × 4.6 mm, 5 μm) (DIKMA, American); acetonitrile-3 % acetic acid (10:90), isocratically eluted at a flow rate of 0.8 mL/min, detection wave at 333 nm and at 25 °C. In fact, this wavelength had ever been adopted to determine sinapine and sinapic acid[5].

**Animal experiment:** Prepare the nutrition of semi-solid paste:10 g starch, 16 g milk powder, 8 g sugar and 2 g carbon powder were added to 36 mL of sodium carboxymethyl cellulose solution (4 %) to achieve a kind of paste for the determination of intestinal propulsion. This paste was stored at 4 °C, heated to room temperature when used[6].

**Gastric emptying experiments:** The method of Gilani and Ghayur[7] was used in this study. Briefly, the mice of each 20-25 g were divided into six groups. Four groups as the test groups were treated with gradient doses of the fresh or cooked juice; one group was the normal control; the sixth group was the positive control. Groups were received gastric administration with aforementioned solutions every day for total 7 days. The saline was for the normal group; domperidone (1 mg/kg b.w.) was for positive control; and the low (90 g/kg b.w) and high (180 g/kg b.w) doses of the fresh and cooked juice were for the test groups. After the last delivery, mice were deprived of food 18 h prior to experiment but allowed free access to water. The groups were received gastric administration of 0.2 mL the nutrition of semi-solid paste, after 0.5 h, the mice were sacrificed by cervical dislocation and the abdomens were immediately opened for obtaining gastric tissue and intestines. When gross weight was measured, the mice were rinsed thoroughly with 80 mL distilled water to get net weight. The gastric emptying rate = [(gross weight-net weight)/semi-solid paste weight of gastric tissue] × 100 %.

**Small intestine propulsion experiments:** The mesentery was isolated from the intestines and the whole small intestine (pylorus region to cecum) was measured. The length of small intestine together with the distance from pylorus region to the front of charcoal meal was measured and then the charcoal transport percentage could be calculated. The carbon power movement rate = [(distance from the pylorus to the farthest carbon power)/total distance from pylorus to ileocecal junction] × 100 %.

**Statistic analysis:** The experimental results were analyzed with SPSS10.0 statistical software and presented as mean± standard error mean (SEM). T-tests were used for statistical analysis. Both $P < 0.05$ and $P < 0.01$were considered to be significant.

## RESULTS AND DISCUSSION

**Sinapine thiocyanate in radish juice:** The standard solution of sinapine thiocyanate was diluted by mobile phase. The method for quantifying sinapine thiocyanate was verified to be reliable by comparing the spectral data of the juice with that of standard solution (Fig. 1). Fortunately, sinapine thiocyanate could be well separated from both the cooked and the fresh juice (Figs. 2 and 3). The calibration curves were achieved by using gradient concentrations of pure sinapine thiocyanate, which was subsequently employed for calculating the concentrations of components in juice. It was a strong negative linear correlation ($R^2 = 0.999$) between the flow rate and the retention time of individual components. The content of sinapine thiocyanate was determined using the standard curve. Sinapine thiocyanate in fresh and cooked juice was 16.66 and 7.43 μg/mL (corresponding to 3.33 and 1.49 μg/g), respectively.

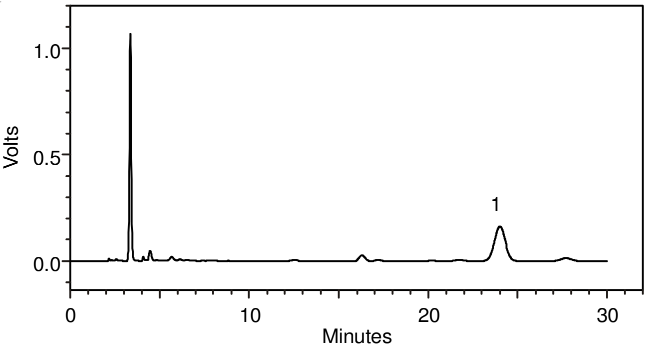

Fig. 1. HPLC analysis of standard solution. The peaks were identified as: 1 = sinapine thiocyanate

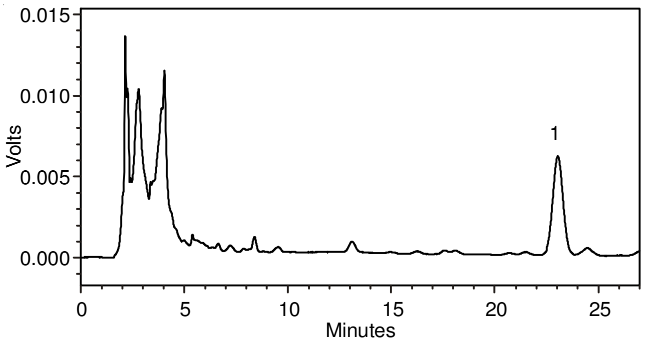

Fig. 2. HPLC analysis of the cooked juice. The peaks were identified as: 1 = Sinapinethiocyanatetec

**Effect of radish juice on gastric emptying:** To investigate the influence of radish on gastric emptying, radish juice was infused into miceonce of everyday, for 7 days. As a result, both the fresh and cooked juice stimulated the gastric emptying and the peaks occurred in the fresh juice group at dose of 180 g/kg (Table-1), the gastric emptying rate in mice was (35.03 ± 2.88) %, indicating that this dose of juice was the most effective. For same dose of radish juice, the fresh juice led to higher gastric emptying rate than cooked juice.

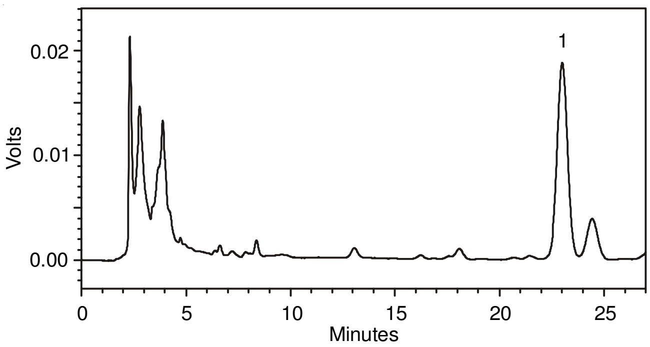

Fig. 3. HPLC analysis of fresh juice. The peaks were identified as: 1 = Sinapinethiocyanate

TABLE-1
EFFECTS OF RADISH JUICE ON MICE GASTRIC EMPTYING

| Group | n | Gastric emptying rate (%) |
|---|---|---|
| Normal | 10 | 26.95 ± 2.41 |
| Fresh juice (180 g/kg) | 9 | 35.03 ± 2.88$^{**}$ |
| Fresh juice (90 g/kg) | 10 | 31.48 ± 3.71$^{**}$ |
| Cooked juice (180 g/kg) | 10 | 28.15 ± 2.45 |
| Cooked juice (90 g/kg) | 8 | 27.70 ± 1.93 |
| Domperidone (1 mg/kg) | 9 | 32.75 ± 2.67$^{**}$ |

$^{**}$In comparison with normal group, P < 0.01

**Effect of radish juice on intestinal movement:** As shown in Table-2, radish juice could propel the charcoal meal crossing the small intestines and the influence on gastrointestinal transit was in dose-dependent mode. This influence was statistically significant. The percentage of crossing distance in the vehicle control (saline) was (45.8 ± 3.9) %, while the cooked juice at the dose of 180 g/kg led to (62.6 ± 5.9) % (P < 0.01). High dose of fresh juice showed distance percentage of (55.7 ± 4.3) % (P < 0.01). For the same dose of radish juice, the cooked juice had stronger impact on gastrointestinal transit.

TABLE-2
EFFECTS OF RADISH JUICE ON SMALL INTESTINAL TRANSIT OF MICE

| Group | n | Distance propelled/ total length of small intestine (%) |
|---|---|---|
| Normal | 10 | 45.8 ± 3.9 |
| Fresh juice (180 g/kg) | 9 | 55.7 ± 4.3$^{**}$ |
| Fresh juice (90 g/kg) | 10 | 50.7 ± 2.7$^{**}$ |
| Cooked juice (180 g/kg) | 8 | 62.6 ± 5.9$^{**}$ |
| Cooked juice (90 g/kg) | 9 | 52.2 ± 6.2$^{**}$ |
| Domperidone (1 mg/kg) | 10 | 66.5 ± 3.3$^{**}$ |

Compared with normal group, $^{**}$P < 0.01

The aforementioned results showed that the sinapine thiocyanate in fresh juice (3.33 μg/g) was higher than that in the cooked (1.49 μg/g), implying that a large amount of sinapine thiocyanate was evaporated or decomposed during the process of cooking. Away from this conclusion, previous work reported that its content in the radish consumed by Korean was up to 41.0 (μg SCN/g dry weight). Clearly, there is a big gap between the two results. It is speculated that the most of sinapine thiocyanate may be deposited in the crude fiber (sediment)[8], or, different extraction methods may lead to diverse results. The third explanation for this may be the difference of radish species or even the outcome arising from plant growth and nutrition[9].

Although radish has been widely regarded as a reliable health food, there are relatively few reports pertaining to the roles of active constituents in ameliorating the gastrointestinal function. Additionally, little is known about the changes of ingredients during the processing of radish. So far, sinapine thiocyanate is known to be the major biologically active component of radish and many studies have shown the obvious effect of radish on antioxidant and radical scavenging activities[10]. In recent years, radish has aroused growing interest in exploring its biological activities. Jeong *et al.*[11] reported that the methyl isogermabull one isolated from the methanol extracts of radish roots could cause a significant increase of the isolated rat ileal contraction in a concentration-dependent manner. In addition, the pattern of MIGB-induced ideal contraction was different in the time course from that produced by acetylcholine[11]. Gilani and Ghayur[7] investigated the effect of crude extract of radish leaves on gastrointestinal motility, revealing a dose-dependent spasmogenicity in guinea-pig ileum and colon. This effect was insensitive to atropine pretreatment but was completely abolished by pyrilamine, indicating the involvement of his taminergic ($H_1$) receptors.

Overall, in the present study, we found that the fresh radish juice contained more sinapine thiocyanate than the cooked. More intriguingly, the cooked juice had a stronger impact on gastrointestinal transit, while the fresh juice exerted significant influence on gastric emptying. Hence, we speculate that the resulting gastric emptying may be in part ascribed to sinapine thiocyanate. In contrast, sinapine thiocyanate exerts little influence on gastrointestinal transit. Although the exact component that stimulates gastrointestinal motility remains to be disclosed, it is evident that sinapine thiocyanate contributes to alimentary gastrointestinal function and it works in dose-dependent manner. For instance, high dose (400 g/kg) of *R. sativus* is toxic to liver cells because it induces hepatocyte necrosis and liver fibrosis[12]. Collectively, sinapine thiocyanate was shown to be an active ingredient, which likely facilitates gastrointestinal motility. In-depth study is required to isolate other components and elucidate their correlation with pharmacological actions.

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