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In vitro model to assess arsenic bioaccessibility and speciation in cooked shrimp

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Abstract

Shrimp, a popular and readily consumed seafood but contains high concentrations of arsenic. However, few studies have focused on whether arsenic in the shrimp could be transformed during the cooking process and gastrointestinal digestion. In this study, a combined *in vitro* model (UBM-SHIME, Unified BARGE Method - Simulator of Human Intestinal Microbial Ecosystem) was utilized to investigate arsenic bioaccessibility and its speciation in raw and cooked shrimps. The results showed that the cooking practices had little effects on arsenic content and speciation. Bioaccessibility of arsenic in raw shrimp was at high level, averaging 76.94±4.28% and 86.657±3.74% in gastric and small intestinal phases, respectively. Arsenic speciation was stable in all of the shrimp digestions, with non-toxic arsenobetaine (AsB) being the dominated speciation. Cooking practice significantly increased the bioaccessibility of arsenate *(P<0.05)* in shrimp digests indicating the increase of the potential health risks~~Compared to raw shrimp, cooking treatments significantly increase the bioaccessibility of arsenic (*P*<0.05) as well as the release of As(V), and increased the potential health risks~~.

Key words: arsenic, shrimp, bioaccessibility, speciation, *in vitro* model

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1. Introduction.

Arsenic is a widely-distributed toxin in the environment. Human are susceptible to arsenic toxicity, and many diseases such as skin lesions, neurotoxicity and even various types of cancers [1, 2] are associated with arsenic exposure. Oral ingestion is the predominant route for arsenic exposure [3]. In addition to drinking water and rice, seafood, including marine animals and seaweed, is also a key source of arsenic dietary exposure [4-6]. Marine shrimp (*Fenneropenaeus chinensis*), a rich source of proteins, omega-3 and vitamin [7], is one of the most popular seafood in the world. Although commonly, relatively large concentrations of arsenic are found in shrimp tissues, consuming shrimp is considered to be safe, as the main arsenic speciation reported in shrimp is non-toxic arsenobetaine (AsB) [8]. However, recent heath assessment studies, have questioned this assumption, and ~~show~~showed a potential hazard associated with highly toxic inorganic arsenic (iAs) content in shrimp [6, 9].

The process of cooking can alter both the total concentration of arsenic in a food, along with speciation and bioavailability [10, 11]. Tawfik et al. [12] have reported that traditional cooking method can increase the arsenic content in shrimp by weight reduction. Moreover, arsenic speciation in shrimp can be ~~change~~changed during thermal treatment. Devesa et al. [13, 14] demonstrated that trimethylarsine oxide (TMAO) and tetramethylarsonium ion (TMA^+) can be formed from the decarboxylation of AsB in shrimp muscle during roasting and baking processes. The toxicity of arsenic is dependent on its speciation~~,~~; thus, the potential risks could be altered due to the

transformation of arsenic species. However, studies also showed that cooking processes have limited effects on the contents of arsenic and its speciation in their edible muscle [15, 16]. So, it is still controvertible whether cooking processes influence the contents of arsenic and its speciation in shrimp.

Traditionally, most health risk assessments associated with oral exposure to arsenic have been conducted using total concentrations, but this at best, provides only a worst case estimate of toxicity because not all the arsenic would be completely released from food during human digestion even in acidic conditions of the stomach [11]. Bioaccessibility, the percentage of the metal(loid) released from its matrix into the gastrointestinal tract (GI) [17], is a more realistic measurement of toxicity since it represents the maximum bioavailability of the metal(loid) from it matrix [18, 19]. Bioaccessibility can be evaluated using *in vitro* models such as PBET (Physiologically Based Extraction Test), IVG (*In-vitro* Gastrointestinal method) and UBM (Unified BARGE Method) [20-22]. For example, Koch et al. [23] used the PBET model to demonstrate the bioaccessibility of arsenic in various food, and showed that the bioaccessibility of arsenic in berries and plants (means of 12%-45%) was lower than that in mushrooms and hare meat (ranging from 22% to 76%). Nevertheless, most of these *in vitro* models still have limitations because they only simulate the digestive processes in the stomach and small intestine, and do not consider the colon environment.

The colon tract has been shownshowed to provide a vast (up to 10^{14} bacterial cells) and diverse (above 1,000 speciation) microbiota, which could contribute to the change of bioaccessibility and its speciation transformation [24-26]. Recently, an *in vitro* model, SHIME (Simulator of Human Intestinal Microbial Ecosystem), cultured with human colon microbiota was developed to evaluate the microbial metabolic potency of ingested substances [25]. By utilizing this model, various arsenic speciation such as highly-toxic monomethylarsonous acid (MMA(III)), dimethylarsinous acid (DMA(III)) and toxicity unknown monomethyl monothioarsonic acid (MMMTA(V)) were found to be associated with colon microbiota [3, 24]. The metabolic activity of human colon microbiota should be considered when assessing human health risks from oral exposure to As. In this study, by using a combined UBM-SHIME *in vitro* models, we aim to (1) assess the effects of cooking methods on the arsenic concentrations and its speciation of shrimp; (2) evaluate the bioaccessibility of arsenic and its speciation changes during gastrointestinal digestion (gastric, small intestinal, and colon phases with human gut microbiota) of the cooked shrimp. The results of this study will give us a more comprehensive insight on potential risks of arsenic associated with cooked seafood.

2. Materials and Methods.

2.1 Chemicals and reagents

Sodium arsenate ($Na_2HAsO_4 \cdot 12H_2O$) (As(V)) and sodium arsenite ($NaAsO_2$) (As(III)) were purchased from BAL (Beijing, China), monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)) were purchased from AccuStandard. Inc (New Haven, CT), arsenobetaine (AsB) stock solution (GBW08670) was purchased from Aokebio. LTD (Beijing China). Ultrapure 18 mΩ·cm water (DDI; Millipore, Bedford, MA, USA) was used throughout all experiments. All the digestive juice (saliva, gastric, duodenal and bile juice) was prepared as detailed in a previous study [21].

2.2 Sample preparation

The shrimp (*Fenneropenaeus chinensis*) used in this study was purchased from a local market in Xiamen city, China. The fresh shrimp was simply washed with running water three times and then divided into three groups. The first group was uncooked as control and the other two groups were boiled and fried, respectively. Cooking processes were carried out in a kitchen gas. ~~For boiling, r~~Running water and soybean oil were used for boiling and frying, respectively. The time for the boiling and frying process was about 4 min. After being cooked, the edible tissues of shrimp were separated from their shells, then frozen dried and ground to a fine powder. All the samples were stored at -20 ^{o}C until use.

2.3 Determination of total arsenic in shrimp

The total arsenic concentrations in both raw and cooked shrimp were determined as previouslyprevious described [27]. Briefly, 0.2 g of freeze-dried edible shrimp samples were placed in high-pressure Teflon tubes with the addition of 4 mL HNO_3 and 0.5 mL HCl, and pre-digested overnight at room temperature in a fume extraction hood. A following three-step microwave digestion procedure was carried out: 5 min ramp from room temperature to 75 ^{o}C and hold for 10 min; 5 min ramp from 75 ^{o}C to 95 ^{o}C and hold for 10 min; 5 min ramp from 95 ^{o}C to 125 ^{o}C and hold for 30 min. After cooling down, the sample solutions were transferred into 50 mL centrifuge tubes and diluted with ultrapure water to a final volume of 40 mL. The solution was passed through a 0.22 μm filter (Millipore, Bedford, MA, USA), and analyzed for arsenic by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500cx, Palo Alto, CA, USA). A Dynamic Reaction Cell (DRC) mode was used to eliminate polyatomic argon chloride interferences, and quantification was performed by internal calibration using germanium (Ge, m/z 72).

2.4 Arsenic extraction and speciation in shrimp

Arsenic species in the shrimp were extracted according to a previous method [28]. Briefly, 0.15 g of freeze-dried samples were accurately weighed and put in the Teflon vessels with the addition of 20 mL of methanol and water (4:1, v/v). The samples were heated and maintained at 95 ^{o}C for 20 min. After cooling, the suspensions were

transferred into 50 mL polyethylene tubes. Next, the samples were placed in a water bath set to 37 ^{o}C, and flushed with nitrogen to remove the methanol. Finally, the extractions were made up with ultra-pure water to a final volume of 20 mL. Arsenic speciation was measured by high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS, Agilent 7500cx, Palo Alto, CA, USA). A Hamilton PRP-X100 anion exchange column (250 mm × 4.1 mm, ID 10 μm particle size, Reno, NV, USA) was used to separate As(III), As(V), DMA(V), MMA(V) and AsB. The mobile phase consisted of a mixture of 10 mM ammonium nitrate and 10 mM diammonium hydrogen phosphate, which had been adjusted to pH 9.25 by ammonium hydroxide [29]. Arsenic speciation in the digestive tract solution was identified by comparing their retention time to those of standards (AsB, As(III), As(V), MMA(V) and DMA(V)), quantified by external calibration curves of DMA(V) [29].

2.5 Dynamic SHIME

The colon microbial community used in the experiment was cultured and maintained in a modified SHIME dynamic model [25]. The SHIME model~~It~~ consists of five double-jacketed vessels kept at a temperature of 37 ^{o}C, simulating the stomach, small intestine, ascending colon, transverse colon and descending colon, respectively (Figure S1). 150 mL SHIME feed (pH 2.0±0.1, arabinogalactan 1.0 g/L, pectin 2.0 g/L, xylan 1.0 g/L, starch 4.0 g/L, glucose 0.4 g/L, yeast extract 3.0 g/L, peptone 3.0 g/L, mucin 1.0 g/L and cysteine 0.5 g/L) was pumped into the stomach vessel, and

150 mL pancreatic juice (pH 6.9±0.2, sodium bicarbonate 12.5 g/L, bile salts 6.0 g/L and pancreatin 0.9 g/L) was pumped into the intestine vessel every day. The three colon vessels were previously inoculated with mixed fecal microbiota obtained from three Chinese adult volunteers without prior antibiotic intake in the six months. The pH values in the ascending colon, transverse colon and descending colon were maintained as 5.6~5.9, 6.1~6.4 and 6.6~6.9, respectively. All of the five vessels were connected one by one, and continuously stirred and kept anaerobic by regularly flushing with nitrogen gas (twice a day, 15 min for each time). After adaptation, we checked both the short chain fatty acids (SCFAs) which is the important metabolites of colon microorganisms and the composition of the colon microbial community in the three colon vessels in the 21st and 27th day. The results showed that the SCFAs concentrations was almost equal (Figure S2) and microorganisms was also almost the same (Table S1) in the two time points. Thus, we confirmed that a stable human microbial community was obtained in the descending colon compartments, and we collected the suspension in colon vessels for further study.~~The three colon vessels were inoculated with mixed fecal microbiota previously obtained from three Chinese adult volunteers who had no history of antibiotic treatment in the six months prior to the study.~~

~~After 4 weeks of adaptation, a stable microbial community was obtained in the descending colon compartments for further study.~~

2.6 Bioaccessibility ~~Bioaccessible~~ of arsenic in shrimp

The *in vitro* model assays used are described previously [21]. Firstly, to stimulate the gastric phase, 0.36 g freeze-dried shrimp powder was accurately added into 100 mL brown serum bottles with 5.4 mL ~~of~~ simulated saliva and thoroughly mixed by manually shaking, and then 8.1 mL of simulated gastric fluid was added. The solution pH was adjusted to 1.3±0.1 with HCl (1 mol/L) and the bottles were capped with rubber stoppers which are impervious to oxygen and flushed with nitrogen gas to keep the anaerobic environment, and shaken (100 rpm) at 37 ^{o}C for 2 h. Subsequently, for the small intestinal phase, 16.2 mL of simulated duodenal fluid and 5.4 mL of simulated bile fluid were added to bottles after 2 h incubation in gastric phase. The pH was adjusted to 6.3±0.5 with NaOH (1.0 M), and the bottles were flushed with nitrogen gas again to ensure the anaerobic environment, then returned to the shaker for an additional 4 h incubation. Finally, in order to simulate colon phase, 35.1 mL of colon SHIME solution (with stable human colon microbota) from the descending compartment of the dynamic SHIME system was supplemented into the bottles after 4 h incubation in intestinal phase. Additionally, nitrogen gas was flushed again to assure anaerobic conditions, and bottles were placed back to shaker and incubated at 37 ^{o}C for an additional 48 h. The experiment was carried out in quadruplicate and destructive sampling was performed. All samples taken from gastric, small intestinal and colon phases were centrifuged at 10,000 g for 10 min, arsenic speciation in supernatant was analyzed by HPLC-ICP-MS. For the bioaccessible arsenic, the supernatant was diluted 10-folds with 0.1 M HNO_3 and analyzed by ICP-MS. Arsenic

in the *in vitro* supernatants was defined as the bioaccessible fraction and bioaccessibility was calculated using the following equation:

$$As\ Bioaccessibility(\%) = \frac{[As]_{filtrate} \times Fluid\ Volume}{[As]_{shrimp} \times Food\ Mass} \times 100\%$$

where $[As]_{filtrate}$ is the total arsenic concentration (mg/L) in the 0.22 μm filtrate, Fluid Volume is the total volume of the gastric, small intestinal and colon fluid (L), $[As]_{shrimp}$ is the total arsenic concentration (mg/kg) in the cooked shrimp, and Food Mass is the total mass (kg) of the shrimp used in the *in vitro* test.

2.7 Quality assurance

To avoid contamination, all glassware, storage bottles and centrifuge tubes were soaked in 10% nitric acid for at least 48 h and rinsed three times with ultra-pure water and preserved dried before use. For total arsenic determination, the certified value of total arsenic in the SRM (GSB-28) sample was 2.34±0.17 mg/kg, indicating a satisfied recovery value of 93.6%. A reagent blank was also included in each batch of sample digestion and analysis to check the precision of the method. In addition, the chromatographic ~~recovery~~recoveries have been validated by dividing the sum of the concentrations of chromatographically detected arsenic speciation by the total arsenic concentration measured by ICP-MS. The recoveries were satisfied 93.~~09~~1%-106.8~~1~~%.

2.8 Statistics

SPSS 16.0 was used for the statistical analysis. A one-way ANOVA and univariate

206 analysis were performed to determine statistical differences. All statistical tests were
207 considered significant at $P<0.05$ level. Results were expressed as mean±standard
208 deviation (n=4). Graphs were performed with SigmaPlot 12.5.
209

3 Results and discussion.

3.1 Concentrations of arsenic and its speciation in raw and cooked shrimp

The average total arsenic concentrations of the raw shrimp sample was 1.37±0.02 $mg \cdot kg^{-1}$ (dry weight). The values for cooked shrimps were similar to raw samples (Table 1), which indicates that the cooking treatments had limited impact on the final arsenic concentrations. While, up to 88.14% of total arsenic in raw shrimp was AsB, which was generally assumed to be non-toxic speciation. It should be noted that 0.14±0.04 $mg \cdot kg^{-1}$ (dry weight) carcinogenic inorganic arsenic was also detected in raw shrimp. The cooking treatments had limited effects (2.19% and 2.92% increase for boiling and frying, respectively) on total arsenic content. Previous studies also showed a similar result in which the total arsenic in cooked seafood was close to the values in raw samples [16, 30, 31]. However, Tawfik et al. [12] showed that arsenic concentrations in cooked shrimp were higher than those found in raw shrimp and it should be noted that those shrimps were cooked with ingredients such as onions, salt and mixed spices which contain some arsenic. Hence, it can be expected that such a study obtained higher arsenic values in the cooked shrimp. In addition, Ersoy et al. [31] reported that arsenic concentration in sea bass fillets was considerably increased from 0.372 $mg \cdot kg^{-1}$ (raw, wet weight) to 2.66 $mg \cdot kg^{-1}$ (fried, wet weight), and the increase generally agreed with the decreased in weight. It is easily to understand that the decrease in weight caused by cooking was resulted from the loss of water and other soluble compounds such as carbohydrates [13], and more importantly the decrease of weight may be the main reason why arsenic was concentrated in cooked

food [15]. However, the shrimp samples (raw, boiled and fried) in our study were cooked without any ingredients, and all shrimp samples were freeze-dried before analysis, so it is reasonable that we did not detect significant changes in cooked shrimp.

Besides the total arsenic concentration, the arsenic speciation patterns between raw and cooked shrimp was also similar (Table 1), with non-toxic AsB accounting for 90.40% and 91.475% of the total arsenic in boiled and fried shrimp, respectively. These results indicate that boiling and frying had no sufficient effects on the speciation changes of arsenic. Our results were consistent with previous study which demonstrated that heat treatment does not produce a change in the arsenic speciation of seaweeds [32]. However, Devesa et al. [13] demonstrated that AsB in shrimp could be decarboxylated to form tetramethylarsonium ion (TMA^+) during roasting, grilling and baking processes. Since a temperature of 150 ^oC was required for transforming arsenic [33], a low liquid temperature (100 ^oC for boiling and less than 140 ^oC for frying) and short cooking time (48 min for both boiling and ~~and less than 5 min for~~ frying) in our study may not be sufficient to catalyze the arsenic transformation. Thus, in order to decrease the toxicological risks from arsenic, low temperature and short cooking time were recommended when cooking shrimp.

3.2 Bioaccessibility of arsenic and its speciation

Arsenic from shrimp exhibited high bioaccessibility values (all above 75.0%) in the digestive tract, which suggests the risk was not generally overestimated. The bioaccessibility of arsenic for raw shrimp upon saliva-gastric juice extraction was up to 76.94±4.28%. The high bioaccessibility values are in agreement with previous studies [11, 34, 35]. Laparra et al. [34] also observed arsenic bioaccessibility between 67.5% and 100% in seafood, which supports our findings. Upon 4 h of intestinal digestion, the bioaccessibility of arsenic significantly (P<0.05) increased to 86.657±3.74% for raw shrimp. The bioaccessibility was higher than reported in other studies [35, 36]. The plentiful active enzyme components, for example pancreatic and bile, used in our study were considered to be the main contributor for the higher bioaccessibility of arsenic in small intestinal digestion than that in gastric digestion [3]. In shrimp, arsenic was mainly distributed in protein and cellular debris [37]. The active enzyme components are efficiently involved in the cleaving denaturalized proteins into free amino acids, the processes could facilitate the releasing of arsenic from protein and cellular debris [38]. Hence, the bioaccessibility was increased from 77.82% to 94.41% for AsB, from 48.53% to 71.61% for As(V) (Table 3). In addition, the longer duration time in intestinal digestion (4 h) than that in stomach (2 h) would also increase the releasing of arsenic from its matrix [39]. For colon digestion, an impressive change of arsenic bioaccessibility in raw shrimp was observed. The arsenic bioaccessibility was decreased from 86.657±3.74% (small intestine 4 h) to 43.20±0.93% (colon 12 h) and then raised back to 95.081±3.09% at the end of simulated digestion. The arsenic

bioaccessibility decrease in colon phase was also detected in a previous study [3], and this may be explained by the binding to the dead microbial biomass that introduced in colon suspension [24]. After 12 hours adaption, the active colon microorganisms showed the ability to breakdown the remaining food matrix , which might further contribute to arsenic release [24]. Therefore, the bioaccessibility of arsenic raised back to a high level. ~~Give such high bioaccessibility, it is reasonable to use the total concentrations to evaluate food safety of arsenic in shrimp~~ if without considering the arsenic speciation.

Furthermore, the bioaccessibility of arsenic for boiled and fried shrimps was significant (P<0.05) higher than that in raw shrimp (Figure. 1). The results implied that cooking could significantly increase the bioaccessibility of arsenic. Interestingly, in stomach stage, the bioaccessibility of As(V) for boiled (58.8~~3~~%) and fried (63.2~~0~~%) was significantly higher than that for raw shrimp (48.5~~3~~%), while there was no significant difference of the AsB bioaccessibility between raw and cooked shrimps (Table 3). Similar to stomach stage, the bioaccessibility of As(V) in small intestinal stage for boiled and fried shrimps was 93.2~~1~~% and 98.~~57~~6%, respectively, which was much more higher than that for raw shrimp (71.6~~1~~%). This might because As(V) was bound to protein in shrimps, and the high temperature during the cooking processing would contribute to the hydrolysis of protein by active enzyme components to release more As(V) resulting in high bioaccessibility [12]. Considering that As(V) is human carcinogen, the increase of the bioaccessibility of As(V) in shrimp after cooking may increase the potential carcinogenic risks.

3.3 Arsenic speciation at colon digestive tract

As human gut microbiota plays an important role in arsenic biotransformation, we also detected the arsenic speciation at colon digestive tract with active colon microbial suspensions. After 48 h incubation with active colon microbial suspensions, only AsB was detected in both raw and cooked shrimp digests, iAs may have become over diluted because the large dilution step in colon stage lead to the concentrations of iAs dropping below the detect limitation (0.2 $\mu g \cdot L^{-1}$ in HPLC-ICP-MS) (Table 2). However, Harrington et al. [40] demonstrated that AsB could be degraded to DMA(V), dimethylarsinoylacetic acid (DMAA) and trimethylarsine oxide (TMAO) after seven days incubation with microorganisms inoculated from human gastrointestinal tract. In order to test the stability of AsB in digestive tract, pure standards of 0.65 $mg \cdot L^{-1}$ AsB solution (500 µL) was add to the digestive juice according to the *in vitro* protocol. We found that AsB was stable presented in gastric, small intestinal and colon phases (~~Figure. 2~~Figure S3). Considering the limited incubation time in our study (48 h with gut microorganisms), no degraded products of AsB in colon phases is quite plausible. The bioaccessible arsenic speciation in both raw and cooked shrimps was stably presented as non-toxic AsB to a great extent in digestive tract.

~~4 Conclusions~~

This study revealed that the bioaccessibility of arsenic in small intestinal phase was higher than that in gastric phase. No degraded products of arsenobetaine was detected in colon phases. Although cooking treatments had limited effects on the changes of

total arsenic ~~total~~ concentration and arsenic speciation ~~changes of arsenic~~ in shrimps, a higher bioaccessibility of As(V) was observed in cooked shrimps, which may increase the potential health risks eventually.

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447

448 **Figure captions**

449 Figure 1 Bioaccessibility of arsenic in raw and cooked shrimp samples. (average ±

450 standard deviation, n=4)

451

452 ~~Figure 2 The chromatography of pure standards of AsB in digestive juice. Arsenic~~

453 ~~speciation was identified by comparing their retention time to those of standards (AsB,~~

454 ~~As(III), As(V), MMA(V) and DMA(V)).~~

455

Tables

Table 1 Arsenic speciation in the raw and cooked shrimp samples. (average±standard deviation, $mg \cdot kg^{-1}$, n=4; Extract efficiency=the sum of speciation/the total arsenic)

	AsB	As(V)	As(III)	iAs	Sum of speciation[b]	Total arsenic [a]	Extract efficiency (%)
raw	1.04±0.05	0.10±0.03	0.04±0.01	0.14±0.04	1.18±0.05	1.37±0.02	85.92
boiled	1.13±0.04	0.09±0.04	0.03±0.01	0.12±0.06	1.25±0.04	1.40±0.03	89.07
fried	1.18±0.07	0.10±0.03	0.01±0.01	0.11±0.04	1.29±0.10	1.41±0.04	91.49

[a] Analyzed by ICP-MS;

[b] Analyzed by HPLC-ICP-MS;

Table 2 Arsenic speciation concentration in gastric, small intestinal and colon phases. (average±standard deviation, μg/L, n=4; ND: not detected; Chromatographic recovery = sum of speciation/total arsenic in supernatant).

		AsB (μg/L)	As(V) (μg/L)	As(III) (μg/L)	sum of speciation (μg/L)	bioaccessibility (%)	chromatographic recovery (%)
stomach	raw	21.58±2.17	1.29±0.21	ND	22.88±2.29	76.94±4.28	93.09
	boiled	24.84±2.26	1.41±0.16	ND	26.26±2.11	83.71±1.94	
	fried	25.23±2.90	1.69±0.22	ND	26.92±3.11	85.94±6.60	
small intestine	raw	10.07±1.08	0.73±0.04	ND	10.81±1.07	86.65±3.74	103.67
	boiled	10.72±0.57	0.86±0.05	ND	11.58±0.60	90.68±4.66	
	fried	11.24±0.21	1.01±0.11	ND	12.25±0.32	92.47±2.77	
colon	raw	5.90±0.06	ND	ND	5.90±0.06	95.08±3.09	106.81
	boiled	6.12±0.17	ND	ND	6.12±0.17	96.44±2.66	
	fried	6.28±0.21	ND	ND	6.28±0.21	98.48±0.62	

ND: Not detectable

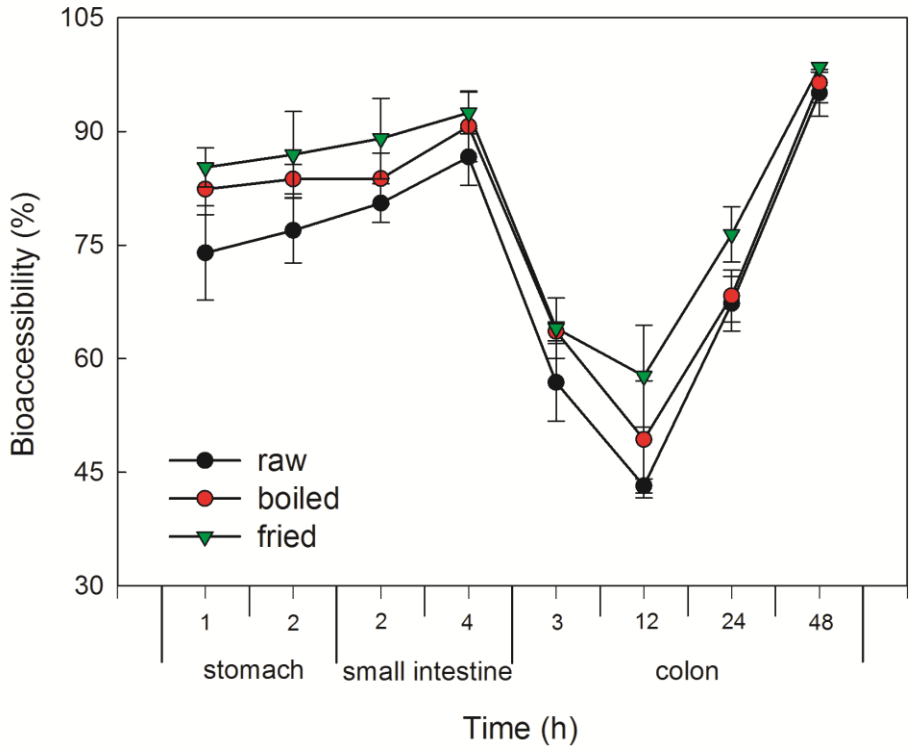
Table 3 Arsenic speciation bioaccessibility in gastric, small intestinal and colon phases.

	stomach (%)			small intestine (%)			colon (%)		
	raw	boiled	fried	raw	boiled	fried	raw	boiled	fried
AsB	77.82	82.45	80.18	94.41	92.51	92.90	87.42	86.74	87.24
As(V)	48.53	58.83	63.20	71.61	93.21	98.57	-	-	-
As(III)	-	-	-	-	-	-	-	-	-

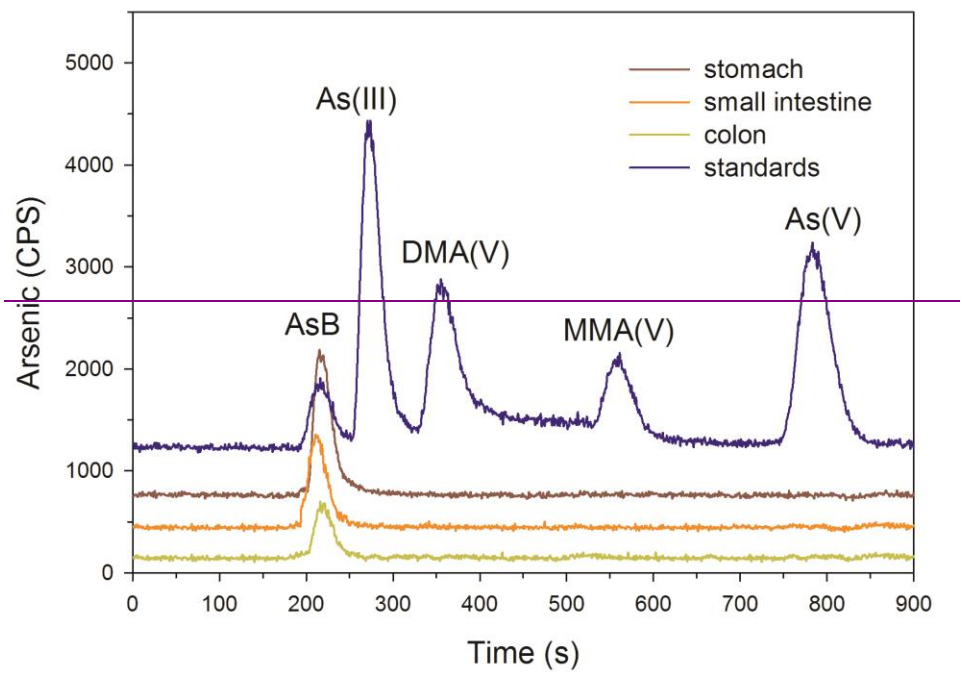
-: no values available.

Figure graphics

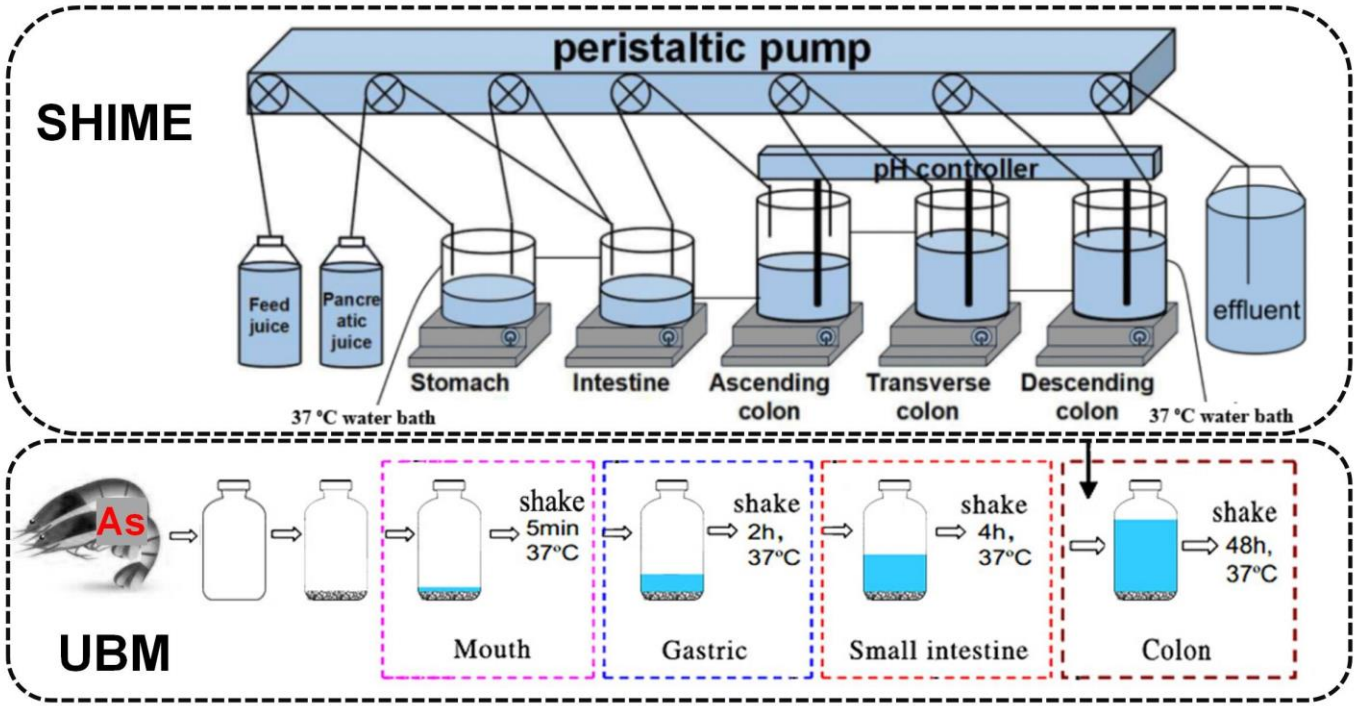
Figure 1

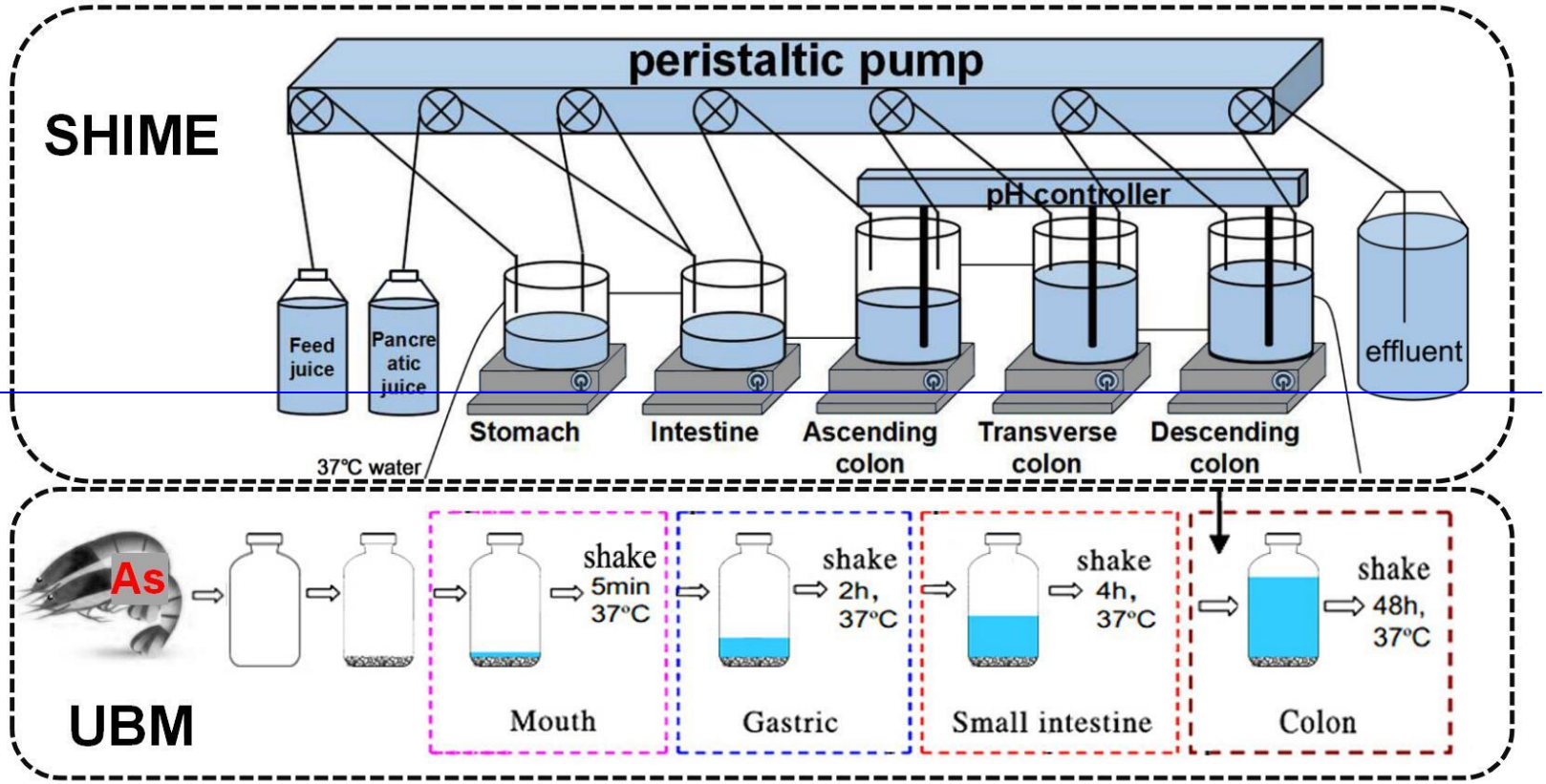


~~Figure 2~~


stomach
small intestine
colon
standards
As(III)
DMA(V)
As(V)
AsB
MMA(V)
Arsenic (CPS)
Time (s)
0
1000
2000
3000
4000
5000
0
100
200
300
400
500
600
700
800
900

Graphic for table of contents




SHIME
peristaltic pump
pH controller
Feed juice
Pancreatic juice
37°C water
Stomach
Intestine
Ascending colon
Transverse colon
Descending colon
effluent
As
shake 5min 37°C
shake 2h, 37°C
shake 4h, 37°C
shake 48h, 37°C
UBM
Mouth
Gastric
Small intestine
Colon