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MiniReview

Bioavailability of heavy metal in rice using *in vitro* digestion model

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Abstract

Rice is a carbohydrate, one of the plant-based foods that can accumulate heavy metal from soil and the irrigation water. Since total heavy metal always overestimates the amount of heavy metal available in rice, bioavailability of heavy metal is always preferred. Many studies have been done and found that *in vitro* methods offer an appealing alternative to human and animal studies. They can be simple, rapid, low in cost and may provide insights which not achievable in the *in vivo* studies. *In vitro* digestion model for rice may differ from other *in vitro* digestion models applied in soil or other type of foods studies. This review aims to provide an overview of *in vitro* digestion model used to determine bioavailability of heavy metal in rice, summarize health risk assessment application of heavy metal in rice studies and highlight the importance of health risk assessment to be included in the studies. Future exploration of *in vitro* digestion model and health risk assessment application on the bioavailability of heavy metal in rice was also suggested.

Introduction

Rice is in carbohydrates group which contains a moderate amount of protein. Rice is also a source of vitamin B, thiamin, riboflavin and niacin (Fresco, 2005). Rice is from the genus of *Oryza* and comprised of 21 species. *Oryza sativa* is believed to have originated from Southeast Asia, while *Oryza glaberrima* is from West Africa. Today, there are thousands of varieties grown and developed originated from *Oryza Sativa* (Kennedy and Burlingame, 2003). Rice (*Oryza sativa* L.) is one of the mostly grown food crops in the world and an important staple food for more than half of the world population (Syahariza *et al*., 2013). Rice has contributed about 40% of the national grain yield in China (Yang *et al*., 2004). Besides that, daily intakes of rice in the Asian countries have reached up to 0.5 kg (dry weight) per caput (Zavala and Duxbury, 2008). Due to it, heavy metal in rice has being concerned and become a serious problem as people take rice as their staple food in daily food. Adults and children may pose a long term health exposure through daily rice consumption (Yang *et al*., 2004). Many studies on multiple heavy metal related with rice have been done worldwide (Mehdi *et al*., 2003; Xu *et al*., 2006; Perello *et al*., 2008; Yap *et al*., 2009; Zhang, 2009; Zhuang *et al*., 2009; Qian *et al*., 2010; Solidum *et al*., 2012). Among the heavy metal that studied, arsenic was found to be the dominant heavy metal found in rice (Bae *et al*., 2002; Laparra *et al*., 2005; Torres-Escribano *et al*., 2008).

Commonly, there are three methods in heavy metal determination namely acid digestion method (aqua regia method, HNO_3 combined with other types of acids such as H_2SO_4, H_2O_2 and $HClO_4$), fractionation of metals into five components (exchangeable, carbonate bound, oxide bound, organic bound, and residual form) and *in vitro* digestion model. Heavy metals using acid digestion and fractionation methods have few limitations as total heavy metal concentration often does not accurately represent heavy metal characteristics and its toxicity (Okoro *et al*., 2012). Moreover, based on Zimmerman and Weindorf (2010), fractionation will have some problems due to the nonselectivity of the reagents and potential for heavy metal to redistribute during extraction process. However, *in vitro* digestion model is preferred rather than acid digestion and fractionation method in the heavy metal determination. *In vitro* digestion model is preferred because it is easy and fast, straightforward, inexpensive, provide accurate results within a short time, reduces the use of experimental animals and the vast amount of different matrices to make it easier to investigate a large number of samples and allowing replication (Oomen *et al*., 2003; Coles *et al*., 2005; Brandon *et al*., 2006; McClements and Li, 2009; Juhasz *et al*., 2010; Chen *et al*., 2011; Hur *et al*., 2011; Yang *et al*., 2012). A current trend of *in vitro*

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digestion model development and utilization for food is evolving. There is still limited number of *in vitro* digestion model for rice as the most predominant food samples tested using *in vitro* digestion model are plants, meats, fish, dairy, and emulsion-based foods (Hur *et al*., 2011).

Looking into the development of *in vitro* digestion model which currently evolving, this review provides an overview of *in vitro* digestion model application studies on the bioavailability of heavy metal in rice. This study also summarized health risk assessment application of heavy metal in rice studies and highlighted importance of health risk assessment to be included in the *in vitro* digestion studies. In simplified term, this new understanding on *in vitro* digestion model will help to give a clear picture and direction of future investigation and exploration of *in vitro* digestion model as well as health risk assessment application on the bioavailability of heavy metal in rice.

In vitro digestion model to determine bioavailability of heavy metal in rice

Studies related to environment and health risk assessment due to heavy metal need a better understanding on the bioavailability of heavy metals in rice. In the aspect of food, bioavailability can be defined as a term that used to describe the proportion of the ingested contaminant in food that reaches the systemic circulation (Versantvoort *et al*., 2005). Total heavy metal in rice does not always reflect the actual amount that is available for absorption by the body. Due to it, bioavailability of heavy metal is preferred in assessing the human health risk.

Bioavailability tests can be divided into two categories which are chemical extraction tests and gastrointestinal analogue tests. Chemical extraction tests are also known as ‘easily extractable metals’ tests. They are simple and are usually at low pH conditions with those that are likely to be bioaccessible. Although the results of chemical extraction tests give a broad idea of easily mobilized contaminants, the extraction conditions and leaching reagents are not representative of those found in the human gastrointestinal tract. Thus, there has been no attempt to calibrate results for this type of test against human *in-vivo* or *in-vitro* studies (Wragg and Cave, 2002). On the other hand, gastrointestinal analogue tests attempts to mimic the biochemical conditions in the human or animal gastrointestinal tract (Wragg and Cave, 2002). There are many types of gastrointestinal analogue tests. There are Physiologically Based Extraction Test (PBET), Simplified Bioaccessibility Extraction Test (SBET), *In vitro* Gastrointestinal Method (IVG), US Pharmacopoeia Method, Mass Balance & Soil Recapture method, German DIN 00 19738, Simulator of Human Intestinal Microbial Ecosystem of Infants (SHIME), RIVM in vitro Digestion Model, TNO Gastrointestinal Model (TIM), and Association of Analytical Communities (AOAC) Pepsin Digestibility Test. Each type of gastrointestinal analogues tests has different physicochemical conditions and stages. Among the gastrointestinal tests, RIVM *in vitro* digestion model is the best model for in vitro digestion model for rice. This model involves three compartments which are oral cavity, stomach and small intestine (Versantvoort *et al*., 2004).

In vitro methods can be simple, rapid, low in cost and may provide insights which not achievable in whole animal studies (Miller *et al*., 1981). Besides that, it reduces the usage of experimental animals. All of these characteristics make it broadly applicable for the human health risk assessment. Most of the *in vitro* digestion models describe a two-step (stomach and small intestine) or three-step procedure (mouth, stomach, small intestine) (Versantvoort *et al*., 2004). Nevertheless, large intestine is not taken into account because human food digestion and absorption mainly takes place in the small intestine and not in large intestine (Oomen *et al*., 2003).

In vitro digestion model has been widely used to study structural changes, digestibility, and release of food components under simulated gastrointestinal conditions (Hur *et al*., 2011). To simulate the gastrointestinal conditions, digestion process in the human gastrointestinal tract was simulated in a simplified manner by applying physiologically based conditions (Yang *et al*., 2012). There are some parameters that influenced physiologically based conditions. The parameters are gastric pH, intestinal pH, food constituents, residence time and particle size (Intawongse and Dean, 2006). Besides, amount of food and contaminants have no effects in the bioaccessibility of contaminants in the *in vitro* digestion model (Versantvoort *et al*., 2005). Yang *et al.* (2012) also found that bioavailability of cadmium from food depends not only on its binding forms but also on the food properties (such as food source and method of processing).

Based on the model described by Versantvoort *et al*. (2005), this model was initially introduced by Oomen *et al.* (2003), simulating the digestive process under a fasted conditions. In order to mimic the fed conditions in the human gastrointestinal tract, there are some changes and modifications have been done and the development of new *in vitro* digestion model for fed condition has been described by Versantvoort

Table 1. Enzymes or chemicals used in different types of *in vitro* digestion model for rice studies

Types of *in vitro* models	Measurements parameter	Enzymes or chemicals	References
In vitro digestion on steryl ferulates from rice and other grains	Steryl ferulates from rice and other grains	α amylase, mucin, BSA, pepsin, pancreatin, lipase, bile salt	Mandak and Nystram, 2012
In vitro digestion model to determine bioaccessibility of cadmium of uncooked rice in a mining area	Cadmium in uncooked rice	α amylase, mucin, BSA, pepsin, pancreatin, lipase, bile salt	Yang *et al.*, 2012
In vitro gastrointestinal fluid system	Arsenic in various type of rice	Pepsin, pancreatin, bile extract,	He *et al.*, 2012
Dynamic *in vitro* digestion model	Bioaccessibility of arsenic in cooked rice	Pepsin	Signes-Pastor *et al.*, 2012
In vitro digestion of rice in HGS by using dynamic stomach model	Physical Changes in White and Brown Rice during simulated gastric digestion	Pepsin, gastric mucin, α-amylase,	Kong *et al.*, 2011
In vitro gastrointestinal digestion of cooked rice	Bioavailability of inorganic arsenic	Pepsin, pancreatin, bile extract	Laparra *et al.*, 2005
In vitro digestion/Caco-2 cell culture model for 15 rice genotype	Iron bioavailability for 15 rice genotypes	Pepsin, pancreatin, and bile extract	Glahn *et al.*, 2002
In vitro digestion model for rice cereal	Optimal Ascorbic acid to Fe ratio in rice cereal	Porcine pepsin, pancreatin, bile extract,	Glahn *et al.*, 1999
In vitro Starch Digestion of Glutinous Rice Flour	Starch digestion in the glutinous rice samples	α amylase, glucoamylase	Zhang *et al.*, 1996

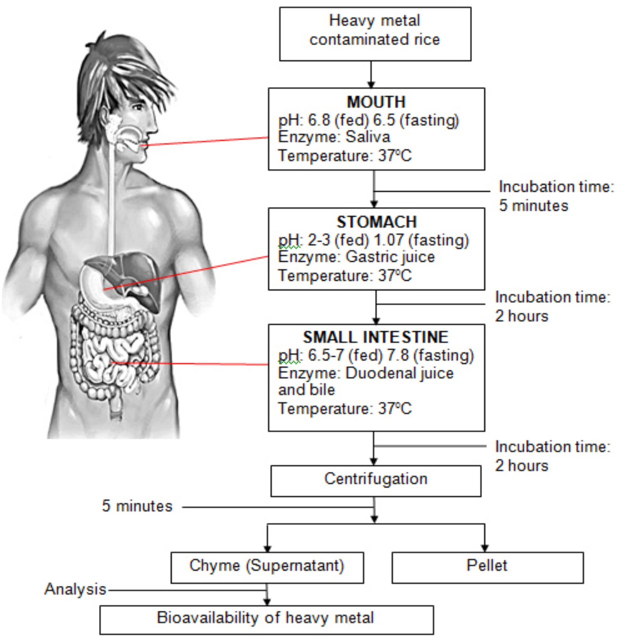

Figure 1. Bioavailability of heavy metal in rice process in the human body

et al. (2004). The changes and modifications may include chemical composition of digestive fluids, pH and residence time periods typical for each compartment. PH may varies depends on the condition such as pH 6.8 (saliva), pH 2-3 (gastric juice) and pH 6.5-7 (duodenal juice and bile) for the fed condition while pH 6.5 (saliva), pH 1.07 (gastric juice), pH 7.8 (duodenal juice) and pH 8.0 (bile) for the fasted condition (Oomen *et al*., 2003; Versantvoort *et al*., 2005; Mandak and Nystram, 2012; Yang *et al*., 2012).

Versantvoort *et al*. (2004) also agreed that an *in vitro* digestion model should fulfill few criteria. The model has to represent physiology of human and last compartment in model must be small intestine, as this is the absorption site of the majority of the compounds. The test also has to be easily applicable, robust and reproducible. Importantly, *in vitro* digestion model describes a three-step procedure simulating the digestive processes in mouth, stomach and small intestine. Firstly, digestion process will be initiated by an addition of artificial saliva to the contaminated matrix. Subsequently, gastric juices and intestinal fluids will be added in order to simulate the digestive processes in stomach and small intestine respectively. The composition of the digestive juices is based on human physiology and will be prepared artificially. Figure I showed the bioavailability of heavy metal in rice process in the human body.

First of all, the mixture in the mouth compartment will be incubated for 5 minutes. Then, the mixture will be incubated for 2 hours at 55 rpm (stomach) and finally the mixture will be incubated for another 2 hours at 55 rpm too (small intestine). At the end of the *in vitro* digestion process, the digestion tubes will be centrifuged for 5 min at 2750 g, yielding the chyme or known as the supernatant. The chyme then will be analyzed for determination of bioavailability of heavy metal in rice. Physiologically, the normal temperature of the human body, 37°C is preferred in the *in vitro* digestion model as it describes the normal temperature of human body (Oomen *et al.*, 2003; Versantvoort *et al*., 2004; Yang *et al*., 2012).

The known and past studies of in vitro digestion model for rice

Table 1 summarizes studies using *in vitro* digestion model for rice. RIVM *in vitro* digestion model was applied by Versantvoort *et al.* (2004), Mandak and Nystram (2012) and Yang *et al.* (2012). These studies focused on fasting condition and fed conditions. Mandak and Nystram (2012) did the *in vitro* digestion study regarding the effect of *in vitro* digestion on steryl ferulates from rice and other grains while the determination of bioaccessibility of

Table 2. Health risk assessment of heavy metal in rice studies

Studied areas	Findings	References
Reclaimed tidal flat soil in the Pearl River Estuary	Health Risk Index (HRI) for all crops exceeds the allowable level considerably and may cause potential health risks. The HRI values of heavy metal acquired mostly from rice as higher daily intake for rice consumption	Li *et al.,* 2012
Around an electroplating plant	No health risk index (HRI) values exceed 1 through rice consumption. Hazard index (HI) through rice consumption for adults and children were 2.075 and 1.808, indicating they may experiences some health effects	Liu *et al.,* 2011
Abandon mine, Thailand	Estimated dietary intake (EDI) for lead were below the tolerable daily intake (TDI) limits while for cadmium is exceed the TDI. Hazard quotient (HQ) value for lead below 1 while cadmium has exceeded	Nobuntou *et al.,* 2010
Industrial zone	Hazard quotient (HQ) values found to be lower than 1 but the hazard index (HI) seem to be close to 1 when combined	Cao *et al.,* 2010
Chinese market milled rice	The children aged 2-14 years old may experience some adverse health effects since the hazard index at the 97.5th estimate were 0.776-1.677	Qian *et al.,* 2010
Rapid industrial area	Cadmium, copper and lead was found to be exceeded their oral reference dose for adults and children. The hazard index (HI) indicated that they may experience some adverse health effects	Hang *et al.,* 2009
E-waste recycling area	Heavy metal such as arsenic, cadmium, mercury, and lead were chosen in health risk assessment	Fu *et al.,* 2008

cadmium of uncooked rice in a mining area has been done by Yang *et al.* (2012). The results found that the bioavailability fraction of cadmium in uncooked rice has a significant positive correlation with total cadmium concentration (Yang *et al.*, 2012). The enzymes involved were same in both study namely α amylase, mucin, BSA, pepsin, pancreatin, lipase, and bile salt but differ in terms of the amount due to the conditions (fed and fasting conditions).

Glahn *et al*. (1999) and Glahn *et al.* (2002) have used *in vitro* digestion model to determine an addition of ascorbic acid to iron ratio in rice cereal by using an in vitro digestion model coupled with caco-2 cell culture too. The findings concluded that an addition of ascorbic acid to rice cereal can increase iron bioavailability in the *in vitro* digestion model (Glahn *et al*., 1999). A dynamic stomach model, known as human gastric simulator (HGS) also has been used in an *in vitro* digestion model done by Kong *et al.* (2011). The study was on the physical changes in white and brown rice and also the effect of them on the gastric fluid properties. The model only focused on the stomach and it was an example of a dynamic model of *in vitro* digestion model

Several studies have been conducted regarding bioavailability of arsenic in cooked rice. The bioaccessibility of inorganic arsenic can increase to 63-99% after the simulated gastrointestinal digestion (Laparra *et al.*, 2005). Another study on the bioavailability of arsenic in cooked rice was based on the effect of arsenic in the cooking water and arsenic content in rice (Signes-Pastor *et al*., 2012). Bioavailability of arsenic from cooked rice using *in vitro* dynamic digestion process based on the static digestion process reported previously by Laparra *et al.* (2005). The study found that parboiled rice showed a higher percentage of arsenic bioaccessibility compared to nonparboiled rice. The results also found that cooking could affect the arsenic content in rice. On the other hand, He *et al.* (2012) did a study on the bioaccessibility of arsenic in various types of rice using an *in vitro* gastrointestinal fluid system. The study found that bioavailability of arsenic in rice depends on the grain itself. Moreover, arsenic bioavailability increases from brown rice, long grain parboiled, long grain and to the highest in extra long grain.

There are few differences between the total heavy metal levels in cooked and uncooked rice due to the effect of cooking. The differences were based on the findings in most studies that the level of heavy metal will increase significantly after cooking. It was due to some chelating effect by rice grains, water contaminated of heavy metal used for cooking and also variety method of cooking (Bae *et al.*, 2002; Laparra *et al.*, 2005; Signes-Pastor *et al*., 2012). Bae *et al.* (2002) suggested that chelating effect by rice grains or a high concentration of heavy metal is because of water evaporation during cooking. These are the factors that cause to high heavy metal concentration in cooked rice compared to uncooked rice (Bae *et al.*, 2002). On the other hand, health risks posed by the presence of arsenic in cooked rice depend on its bioavailability from the matrix along the digestive system (Signes-Pastor *et al*., 2012). Signes-Pastor *et al.* (2012) observed that cooking water contaminated with high arsenic content increased arsenic concentration in cooked rice compared to uncooked rice.

Health risk assessment application

Table 2 summarizes the health risk assessment application involving heavy metal in rice studies. Unfortunately, there is still limited application of health risk assessment in vitro digestion model using rice studies. It may due to a complex chain from the primary agricultural products until the food being taken by individuals. So, this makes some difficulties to assess the actual health benefits and risks directly in food consumption. Health risk assessment in rice through ingestion pathway was chosen because rice is considered as one of the foodstuffs that have high ingestion rate with high self-planted rates (Cao *et al.*, 2010).

Most of the health risk assessment studies based on rice have been done in China, where the study locations are near to the mining area and e-waste recycling areas (Fu *et al.*, 2008; Hang *et al.*, 2009; Zhuang *et al*., 2009; Cao *et al.*, 2010; Qian *et al.*, 2010). There was also a study done around an electroplating plat in China and at the reclaimed tidal flat soil in the Pearl River estruary, China (Liu *et al*., 2011; Li *et al*., 2012). Both studies applied Health Risk Index (HRI) to characterize the risk to human health. Another study found in an abandoned mine in Thailand to determine the potential health problems in the downstream communities (Nobuntou *et al*., 2010). *In vitro* models of the human gut offer a fast and reproducible methodology rather than total heavy metal to be used in health risk assessment (Wiele *et al.*, 2007). Thus, limited studies on health risk assessment application have proved that *in vitro* digestion model was seen as an aid to a better and accurate exposure assessment of contaminants for food especially rice (Lee *et al.*, 2006).

Since public health risk associated with dietary intake of heavy metal increased, health risk assessment is crucial to be done in order to detect the heavy metal contamination from food. It is essential to decrease heavy metal accumulation especially in plant-based food especially in rice as it is a main staple food for half of people worldwide. Health risk assessment is very useful so that we can find prevention, cure, and control efforts toward the sources of heavy metal contamination. Thus, it is vital to include health risk assessment in the *in vitro* digestion studies (Versantvoort *et al*., 2004). Based on Versantvoort *et al.* (2005), exposure assessment to a contaminant from food is calculated based on the sum intake (external exposure) of contaminant per food product rather than on the total internal exposure, whereas the internal exposure is correlated with toxicity of the contaminant. Human health risks assessment will be overestimated when total heavy metal concentration is taken into account (Yang *et al*., 2012). Moreover, through the substitution of total concentration with bioavailability of concentration, health risk calculations proved more a realistic result indirectly facilitating an improved cost-benefit analysis of site remediation options (Williams *et al*., 1998). Hence, a bioavailability of less than 100% implies low internal exposure to the contaminant (Versantvoort *et al*., 2004).

Conclusion

This paper reviewed *in vitro* digestion model in determining the bioavailability of heavy metal in rice. As total heavy metal always overestimates the level of heavy metal, *in vitro* digestion model has been chosen to be the best method to assess the bioavailability of heavy metal in rice. An *in vitro* digestion model that proposed by Versantvoort *et al*., (2004) which is the RIVM *in vitro* digestion model is the best used in the determination of bioavailability of heavy metal in rice since it mimics quite close to the physiological condition in human body. Previous studies that have been done using the digestion model can be a reference for a further study to study the bioavailability of heavy metal in rice. Limited studies have applied health risk assessment using *in vitro* digestion model output (bioavailability of heavy metal). Health risk assessment is essential to be included in the *in vitro* digestion studies as a rapid indication of heavy metal contamination and potential human health indicator. This mini review is beneficial especially to Asian people since rice is the main staple food for Asian. As a final note, this review pointed out a good understanding and reference of all *in vitro* digestion studies using rice with suggestions the importance of health risk assessment application in future *in vitro* digestion model studies.

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