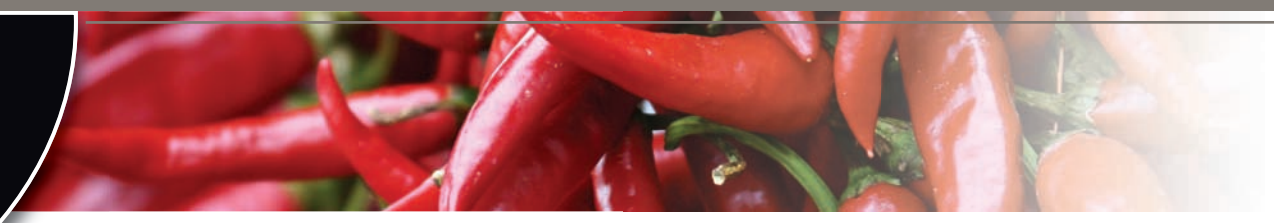

Sample Preparation and Analysis of Arsenic in Rice

Peter Jackson, Sales Director Retsch SEA, Tel: +66 8 1348 7569, Email: p.jackson@retsch.com

There have been a number of recent reports published concerning the levels of arsenic found in rice and rice products [1-3]. Consumer Reports in the United States is recommending that people limit their rice consumption, after finding arsenic in over 60 rice products they tested [1]. The group is urging the US government to set limits on arsenic in rice, just like it does for drinking water, where the Maximum Contaminant Limit (MCL) for total arsenic is currently set at 10µg/L by the US Environmental Protection Agency (EPA) [4].

Sources of Arsenic

Arsenic is a chemical element widely distributed in the Earth's crust. It is released from volcanoes and from the erosion of mineral deposits and is found throughout the environment in water, air and soil. Human activities also add arsenic to the environment. They include burning coal, oil, gasoline and wood, mining, and the use of arsenic compounds as pesticides, herbicides and wood preservatives.

Arsenic is a contaminant and a known carcinogen which exists in both toxic and non-toxic forms. It occurs in inorganic and organic compounds; as trivalent (III) and pentavalent (V) oxidation states; and as anionic, cationic, and neutral species. In general, the methylated and other organoarsenic compounds are less toxic than inorganic arsenic, and pentavalent arsenic is considerably less toxic than the trivalent state [5].

While most crops don't readily take up much arsenic from the ground, rice absorbs arsenic from soil or water much more effectively than most plants. This is primarily because it is grown in water-flooded conditions, which allow arsenic to be more easily taken up by its roots and stored in the grains [1]. Rice is grown all over the world and is grown differently from region to region, hence the levels of arsenic within the same kind of rice product may well be expected to vary by geographic region.

Consumer Reports also found that the average total level of inorganic arsenic was higher in brown rice than in white rice and where they tested both a white and a brown rice of the same brand: the inorganic arsenic levels were higher in the brown rice than in the white rice in all cases [1]. Arsenic is known to concentrate in the outer layers of a grain, so it is perhaps not surprising that brown rice might have higher levels of arsenic, as the process of polishing rice to produce white rice removes the surface layers, hence reducing the total arsenic in the grain. In brown rice, only the hull is removed. Arsenic concentrations found in the bran that is removed during the milling process to produce white rice can be 10 to 20 times higher than levels found in bulk rice grain [1].

Sample Preparation of Rice

Considerable care must be taken when analysing a sample like rice in order to achieve an accurate result. The major source of error when analysing a bulk material comes not from the analytical measurement itself, but from the sample handling, such as, sampling, sample division, grinding and digestion. [6]. Rice particles are subject to segregation as the bulk sample is a mixture of whole and broken grains with different sizes. Random sampling from a bulk material can contribute up to 10% variation in the final result. The use of automated rotary tube dividers, such as Retsch's PT 200, can reduce the variation resulting from the sampling process to 1% or less [6].

Once a representative bulk sample is taken, the rice must be ground to a suitable size range. The grinding process serves firstly to homogenise the sample, while the subsequent sample preparation steps or analysis method typically require a certain fineness of material. Different types of analysis require different methods of sample preparation and different particle size ranges. For the majority of digestion procedures, the particle size should not be so fine that the reaction occurs too quickly and if the material is too coarse, the digestion process takes too much time. In general, particle sizes around 500 microns are optimal for sample digestion. The particle size is of similar relevance for extraction methods. If the powder is too coarse, not everything can be extracted and if the powder is too fine, the extraction thimble can be blocked and the material is flushed into the receiver flask. While the optimum fineness of the sample strongly depends on the extraction apparatus used, for most systems, around 500 microns is an optimal size.

If the material is analysed directly, then the required fineness depends upon the instrumental technique. For NIR analysis the particle size should be around 500 microns to ensure that the light completely penetrates the particles, while for pelletised material to be analysed by XRF, the optimal particle size is in the order of 40 to 60 microns. *Table 1* illustrates the recommended analytical fineness for a number of instrumental techniques.

Table 1. Recommended analytical fineness for a variety of instrumental techniques

Technique	Sample type	Analysis	Fineness
AAS	Ores, rocks, plants	Elemental analysis	20 – 30µm
ICP, ICP-MS	Ores, rocks, plants	Elemental analysis	20 – 60µm
XRF	Metals, ores	Elemental analysis	40 – 60µm
FTIR	Raw materials, pharmaceuticals	Organic compounds	50 – 100µm
DTA	Plastics, refuse	Calorific value	200 – 300µm
NIR	Feeds, grains	Protein, fat, moisture	200 – 500µm

Rice can be ground using an Ultra Centrifugal Mill, such as the RETSCH ZM 200, prior to analysis. The ZM 200 is a high speed rotor mill and the size reduction takes place by impact and shearing effects between the rotor and a fixed size ring sieve. The feed material only remains in the grinding chamber for a very short time, which avoids the risk of overheating and ensures that the characteristics of the sample to be analysed remain unaltered. A sample of 100 grams of rice (3 – 7mm particle size) can be completely reduced in size to below 100µm using a ZM 200 operated at 18,000 rpm with 24 tooth push-fit rotor and a 0.12mm distance sieve plus cyclone with passage receptacle and holder in under 3 minutes [7]. Smaller particle sizes can be obtained by changing to a ring sieve with smaller aperture sizes.

Figure 1. Retsch ZM 200 Ultra Centrifugal Mill for rapid grinding of rice, feed size 3-8mm, down to a size range of 60 – 100µm in minutes

Rice can also be ground using a Retsch CryoMill, which features an integrated liquid nitrogen cooling system ensuring that the grinding jar is continually cooled before and during the grinding process. The cooling is important for the determination of the species of arsenic in a rice sample. Arsenic occurs primarily in two inorganic forms, arsenite, As (III), and arsenate, As (V). The valence of arsenic is not stable and easily oxidised by heating. Therefore, it is important to grind samples under low temperature prior to speciation analysis [8]. A 10 gram sample of rice can be ground in a CryoMill using 2 x 150 second grinding cycles at 30 Hz to produce a powder with a D50 of 32 microns. The CryoMill also improves the speed of the grinding process by a factor of two compared to grinding at ambient temperature as the low temperature embrittles the rice grains making them easier to fracture and pulverise [8].

Figure 2. Retsch CryoMill for cryogenically cooling the rice sample during size reduction prior to arsenic speciation analysis

Analysis of Arsenic in Rice

The analysis of total arsenic is most commonly achieved using acid digestion followed by a spectroscopic detection method such as hydride generation atomic absorption spectrometry (HG-AAS), or hydride generation atomic fluorescence spectrometry (HG-AFS) [9]. The more recent use of hyphenated analytical techniques such as ICP-OES and ICP-MS allows the determination of arsenic without the need for hydride generation. In order to determine the individual arsenic species, such as As (III), As (V), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and arsenobetaine (AsB), in a sample, the spectroscopic detector must be coupled with a chromatographic separation technique such as ion chromatography or high performance liquid chromatography [5].

Outlook

Many studies of arsenic exposure from drinking water in numerous countries have shown that it causes lung and bladder cancer and other diseases [10]. While there is no documented evidence of actual adverse health effects from exposure to arsenic in rice, the US FDA understands that consumers are concerned about arsenic being in rice. The FDA is currently conducting a study of the levels of arsenic in about 1,200 rice products. Once the study is completed, the agency will conduct a comprehensive risk assessment to determine how much risk is associated with long-term consumption of rice and whether it might be necessary for people to adjust their rice consumption.

Conclusions

An accurate analysis is closely linked to correct sample handling. Rotating sample dividers and rotary tube dividers are an important means to ensure a representative sample of a material such as rice. The rice can then be milled in order to achieve the required homogeneous analytical fineness for subsequent analysis. The Retsch ZM 200 Ultra Centrifugal Mill is suitable for the rapid milling of rice samples up to 5 litres in volume. A sample of 100 grams of rice can be reduced to a size below 100 microns in under 3 minutes using this machine.

Alternatively, smaller samples of rice can be ground using a Retsch CryoMill which continually cools the sample during the grinding process. The cooling is important for the speciation of arsenic in a rice sample as the valence of arsenic is not stable and easily oxidised by heating. Both of these sample preparation approaches have been successfully used in the analysis of arsenic in rice by inductively coupled plasma mass spectrometry (ICP-MS) and by ion chromatography coupled to ICP-MS for arsenic speciation.

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DNA Test Identifies Eight Animal Species in Foodstuffs Quickly and Reliably

With horsemeat in lasagnes and pork in kebabs, the meat scandal is escalating day by day. Consumer protection groups and politicians are now calling for Europe-wide DNA testing for a whole range of foodstuffs.

Using the CarnoCheck DNA test, hundreds of samples are currently being tested at various European laboratories as part of efforts to find answers in the horsemeat la-belling fraud. **Greiner Bio-One** has been offering the CarnoCheck DNA test kit since 2004, enabling eight animal species to be detected in foodstuffs quickly and reliably. As well as horses, the kit covers pigs, donkeys, sheep, cattle, chickens, turkeys and goats.

Greiner Bio-One, based in Frickenhausen, specialises in developing molecular biol-ogy analysis methods. Using 'DNA chip technology', which is already deployed by countless laboratories, the specific genetic fingerprint of many different parameters can be detected unambiguously in one single analysis. With the CarnoCheck DNA chip, the eight animal species can be identified in foodstuffs down to a detection limit of 0.5%. The results are available within three hours. A comprehensive control system also ensures the quality of the analysis.