

Samples:

The following document summarizes the acrylamide seven-day extraction data acquired for fourteen different water bead samples received by LSC, completed by 09/15/2023.

Samples had a varied number of bead sizes. In total there were 24 different subsets that required extraction (e.g., one sample may have two subsets – medium and large). Three trials were conducted for each subset of water beads in each sample. A total of 72 bead extraction trials were performed.

There were four subsets of beads: small (hard), medium (hard), large soft, and large hard. The distinction between “small” and “medium” beads was not always clear. For our uses, we considered “small” beads to be ≤ 2 mm in diameter prior to hydration, “medium” beads to be >2 and <3 mm in diameter prior to hydration, and large beads ≥ 3 mm prior to hydration. When it was difficult to measure the bead size accurately, a further confirmation of “small” vs “medium” was determined by putting the beads through the extraction method and determining the maximum number of beads that would fit in the digestion vessels with the given volumes of solution. If the number of beads was above 50, it was labeled a “small” sample. If the number of beads was 50 or below, it was labeled a “medium” sample.

Extraction method:

The basic extraction method was as follows, with modifications performed as individual samples and staff’s working hours required.

A multi-day extraction was performed to determine the amount of extractable acrylamide in water beads using a simulated saliva solution (tap water, 5mL), a simulated stomach acid solution (0.07 mol/L HCl, made in tap water, 10-20 mL), and a simulated small intestine fluid (tap water with pH adjusted to approximately 7.4, 45-50 mL). Small and medium beads were tested in sets of 30-100 beads, depending on how many could fit in the vessel with the amount of solution required, while still having solution left over to take aliquots for testing. Large beads, both hard and soft, were tested individually.

Each set of water beads were taken through an extraction process to simulate how they would travel through a human digestive tract. The beads were extracted first in simulated saliva (tap water, 5 mL) at room temperature with no shaking for five minutes. The beads were then transferred to a simulated stomach acid ((0.07 mol/L HCl, made in tap water, 10-20 mL) at 37 °C shaking at 60 RPM for two hours. Finally, the beads were transferred to a simulated small intestine fluid (tap water with pH adjusted to approximately 7.4, 45-50 mL) at 37 °C shaking at 30 RPM where they were left for up to seven days. Aliquots of the extraction solutions were taken at various time intervals as work schedules permitted (after 5 minutes in saliva, 2 hours in stomach acid, and 6, 24, 48, 72, 96, and 168 hours in small intestine fluid) and analyzed for acrylamide using a LC-TQMS instrument.

Analysis:

Concentrations of acrylamide as well as mass of acrylamide extracted were determined for each trial at each time interval. The concentrations of acrylamide were given by the instrument. The mass of acrylamide extracted was calculated from the concentration of acrylamide and the calculated volume of solution that remained when the aliquot was taken.

Major results:

For all beads tested, at most of the time intervals, acrylamide concentrations >1 ppb were found in each extraction solution. One sample showed acrylamide concentrations <1 ppb after the 2 hour in stomach acid interval. A few trials showed acrylamide concentrations <1 ppb after 168 hours in small intestine fluid.

The table below shows, for each trial, the time interval that had the highest extracted acrylamide (mass) measured. It is broken down by bead type. None of the bead trials had a maximum extracted acrylamide value in the 72, 96, or 168 hour time interval.

Bead type	Trials with highest extracted acrylamide (mass) at 5 min		Trials with highest extracted acrylamide (mass) at 2 hours		Trials with highest extracted acrylamide (mass) at 6 hours		Trials with highest extracted acrylamide (mass) at 24 hours		Trials with highest extracted acrylamide (mass) at 48 hours	
	#	%	#	%	#	%	#	%	#	%
Small	2	7	11	41	3	11	0	0	11	41
Medium	0	0	9	50	9	50	0	0	0	0
Large, hard*	0	0	19	95	0	0	1	5	0	0
Large, soft	0	0	6	100	0	0	0	0	0	0
Total (of 71 trials)	2	3	45	63	12	17	1	1	11	15

*one large, hard bead trial was excluded because the extraction solution was lost at 6 hours.

For about half of the extraction trials, the extracted acrylamide (μg) increased from 5 minutes to 2 hours, decreased from 2 to 6 to 24 hours, increased from 24 to 48 hours, then decreased from 48 to 72 to 168 hours. The large, hard beads followed this pattern the most often. About 80% of large, hard beads followed this pattern.

The highest amount of extracted acrylamide found at any one time interval, for sets of small beads, was 21.971 μg , measured at the end of the 6 hours in small intestine fluid. The highest amount of extracted acrylamide found at any one time interval, for sets of medium beads, was 1.386 μg , measured at the end of the 6 hours in small intestine fluid. The highest amount of extracted acrylamide found at any one time interval, for a large, hard bead, was 14.478 μg , measured at the end of the 2 hours in stomach acid. The highest amount of extracted acrylamide found at any one time interval, for a large, soft bead, was 1714.72 μg , measured at the end of the 2-hour stomach acid extraction. The two large, soft bead samples both had significantly more extracted acrylamide than the other twelve samples.