

# High-Throughput Homogenization of Grain Samples for Deoxynivalenol (DON) Testing



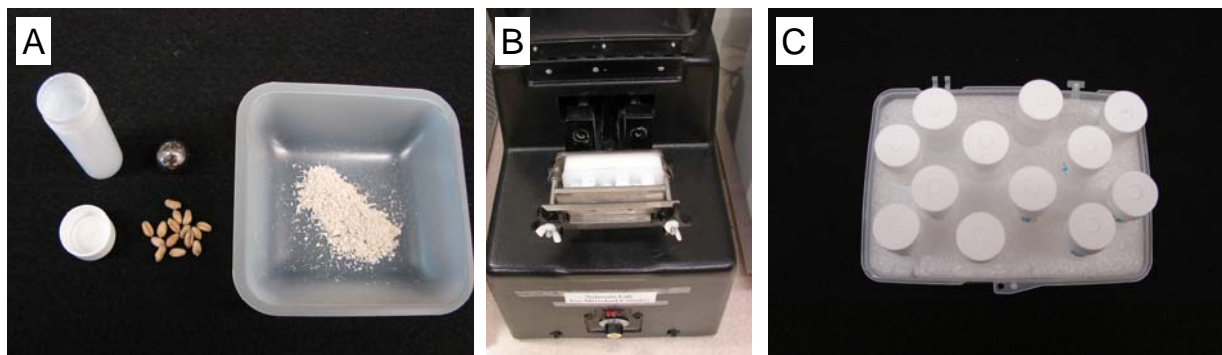
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## ABSTRACT

Concerns about deoxynivalenol (DON) continue to mount, and there is a growing need to develop new tools and techniques to enhance the speed, capacity, and uniformity of DON testing services in the United States. Tens of thousands of wheat and barley samples associated with USWBSI research projects are processed by DON testing labs every year. Many of these samples consist of 100 g kernel lots, and they must be cleaned, milled, and sieved before DON is extracted and quantified. The processing of a high number of grain samples in such a manner is extremely laborious and costly. We developed a rapid and affordable high-throughput homogenization protocol for DON testing that can homogenize twelve grain samples weighing from 0.1 to 2.5 g in as little as ten seconds. A Biospec MiniBeadBeater-96™ operating at 2100 oscillations per minute was used to homogenize grain samples in individual 7 mL HDPE vials containing 13.7 mm chrome balls. Grain samples were homogenized into a fine flour of nearly uniform particle size, and DON extractions were conducted in the same vials that were used for the homogenization of the samples. The extraction solvent containing DON was passed through a clean-up column, and a measured fraction of the flow-through was dried down using a nitrogen evaporator at 55°C. DON samples were derivatized using TMSI and quantified using a GC/MS operating in a SIM/SIM scan mode for target and reference ions of DON. Over 800 grain samples originating from single inoculated or non-inoculated (control) wheat spikes from southern uniform FHB greenhouse trials in AR, NC, and VA were processed for DON testing using this new methodology. High-throughput homogenization protocols may assist in providing affordable and timely DON testing services for USWBSI-associated research projects in the future.



**FIGURE 1.** Grain samples collected from individual greenhouse-inoculated wheat spikes were homogenized in individual 7 mL HDPE vials containing 13.7 mm chrome balls (A). A Biospec MiniBeadBeater-96™ operating at 2100 oscillations per minute was used to homogenize the samples into a fine flour of nearly uniform particle size (B). Twelve grain samples weighing from 0.1 to 2.5 g were homogenized in as little as ten seconds (C).

## INTRODUCTION

- ❖ Every year, tens of thousands of wheat and barley samples associated with USWBSI research projects are processed by DON testing labs.
- ❖ Sample cleaning, milling, and sieving of grain samples is laborious and costly.
- ❖ Many USWBSI investigators routinely screen new lines of wheat and barley in the greenhouse for FHB resistance. The small sample weight of these grain samples poses a number of challenges for accurately detecting and quantifying DON.
- ❖ The goal of this work is to develop a high-throughput homogenization protocol to assist in providing affordable and timely DON testing services for USWBSI-associated research projects.

## MATERIALS AND METHODS

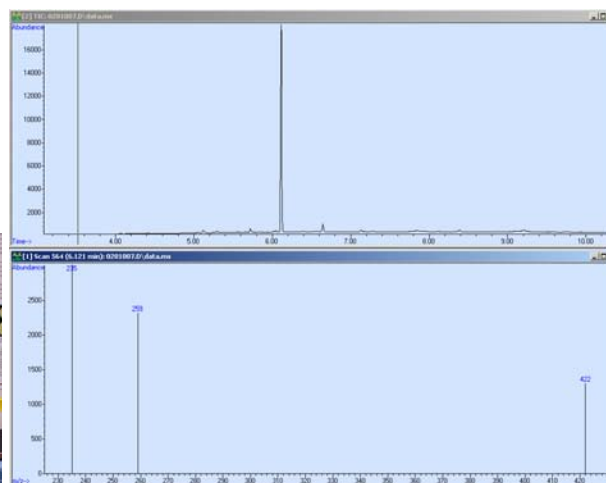
- Wheat spikes were obtained from Southern Uniform FHB trials conducted in AR, NC, and VA. Grain was threshed from individual spikes, weighed, and transferred to 7 mL HDPE vials containing 13.7 mm chrome balls.
- A Biospec MiniBeadBeater-96™ operating at 2100 oscillations per minute was used to homogenize grain samples [FIGURE 1].
- DON extractions were conducted in the same vials that were used for the homogenization of the samples. DON clean-up, dry-down, and derivatization was conducted following standard protocols.

## RESULTS AND DISCUSSION

1. Over 800 grain samples originating from single inoculated or non-inoculated (control) wheat spikes from southern uniform FHB greenhouse trials in AR, NC, and VA were processed for DON testing using this new methodology.
2. Twelve grain samples weighing from 0.1 to 2.5 g were homogenized in as little as ten seconds [FIGURE 1].
3. DON was quantified using a GC/MS [FIGURE 2] operating in a SIM/SIM scan mode for target and reference ions of DON [FIGURE 3] with a detection limit of 0.01 ppm. DON levels ranged from 0 ppm to > 20 ppm.
4. High-throughput homogenization protocols may assist in providing affordable and timely DON testing services for USWBSI-associated research projects in the future.



**FIGURE 2.** DON was quantified using a GC/MS (Agilent 5975 Inert MSD with 6890N Network GC system) operating in EI mode.



**FIGURE 3.** DON was quantified using a GC/MS operating with a SIM/SIM scan for target and reference ions of DON. DON is shown in the top figure with a peak at a retention time of 6.1. The target (235) and reference (259 and 422) ions of DON are shown in the bottom figure following a SIM/SIM scan.