

SEGREGATION OF SIMULATED RFID MARKERS DURING HANDLING AND TRANSPORT OF WHEAT

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ABSTRACT. *The ability to mark bulk goods from different origins with RFID markers is of industrial interest, as such a method would improve the traceability of, e.g., cereals. However, due to a number of open technical questions, this method has not been utilized on a larger scale yet. This article studies the amount of segregation occurring between RFID markers, which are simulated as grain-sized plastic capsules, and marked wheat using two different setups. This segregation could occur during handling and transport due to the slightly different physical properties of the markers and the grains; it would then lead to erroneous results during subsequent quantitative analysis. In the first experiment, two samples of wheat, one marked with RFID dummies, were discharged in several steps from a test silo. A comparison of the marker concentration in the samples with the amount of associated wheat showed no discernible segregation. An additional statistical analysis allowed us to establish a relationship between the marker concentration and the error margin. In the second experiment, a mixture of wheat and markers was vertically shaken in a container, mimicking transport of wheat in large vessels. The position of the markers inside the container was determined by three-dimensional scans using x-ray tomography. We found that shaking induced some segregation due to sidewall-driven convection rolls, which indicated that the simulated markers were not optimally matched to the wheat grains.*

Keywords. *Bulk good, Computed tomography, Marking, Shaker, Silo, Traceability.*

The direct marking of bulk goods is an alternative method to conventional traceability systems, especially in the agricultural sector, where it can, e.g., help to distinguish between different batches of cereals (Hirai et al., 2006). Radio-frequency identification (RFID) markers can actively link information (stored on the RFID chip encapsulated in the marker) with the bulk goods in which they have been inserted. This would improve the documentation of cereals during the transport from their origin to their processing point (Beplate-Haarstrich, 2008; Hirai et al., 2006; Hornbacker et al., 2011; Sui et al., 2007). The biggest advantage of RFID markers over the conventional system of documenting the transport chain is the precise knowledge of the real ratio of cereals from different origins in a given batch. Especially in case of food-borne hazards, this would make remedial measures significantly simpler and cheaper (Trienekens and van der Vorst, 2006). The first published number of markers per cereal charge can be found in Hirai et al. (2006).

Based on logistic considerations, the authors suggest five markers per kg cereal.

Segregation (demixing) of the markers inside the sample would pose a major problem for any quantitative analysis. Segregation can originate from differences in a number of physical properties, such as size, density, form, and surface roughness (Schulze, 2006). If these differences become large enough, the grains and markers will demix as soon as the sample is mechanically excited, e.g., through vibration during transport or when subjected to shearing forces during decanting (Ottino and Khakhar, 2000; Schröter et al., 2006). Consequentially, the relevant physical properties of the markers should be as identical to the bulk material as possible. A first study on segregation was conducted by Beplate-Haarstrich (2008), who tested eight different types of markers (referred to as corn dummies), which differed in their form and specific density. The marker found to evince the smallest amount of segregation (corn dummy type 5) had a smooth surface and a density of 1.378 g cm^{-3} . This marker, which did not contain an actual RFID chip, was also used in this study. As the RFID chip would not change any surface property of the marker and would cause only been a very minor modification of the density, the segregation behavior of the simulated marker is considered representative of the RFID marker.

In this study, we first investigated the reliability of RFID marking of cereals during handling, or more precisely, decanting from a silo. The rationale is that silos are where the greatest degree of admixing of different batches occurs (Schwedes, 2006). Our objective was to test, using statistical analysis, if the amount of markers found in the outflow was a fair description of the sample composition. We found

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that the accuracy of this method was indeed limited only by statistical fluctuations, which demonstrated an absence of segregation in this setup.

In the second series of experiments (shaker experiments), a wheat-marker mixture was shaken vertically on a vibration exciter in order to investigate if segregation occurs when such mixtures are subjected to the vibrations expected during standard transportation processes (Hinsch et al., 1993; Mitschke and Wallentowitz, 2004). The three-dimensional position of the markers both before and after the shaking was determined using x-ray tomography. From the spatial distribution of the markers after shaking, we concluded that some amount of segregation due to sidewall-driven convection rolls had occurred (Schröter et al., 2006).

MATERIALS AND METHODS

MATERIALS

Wheat was used as the test bulk material for the experiments described in this article, as it is a very important cereal globally with respect to the quantity harvested and the area cultivated (Kersten et al., 2004; USDA, 2011). To prevent inhomogeneity, wheat from a single batch was used for all the experiments, namely winter wheat (*Triticum aestivum* L.) of the cultivar *Kranich* (Lantmännen SW Seed, Sweden) harvested in 2009. The batch of wheat was cleaned using a sieving machine produced by Petkus Technologie GmbH (Wutha-Farnroda, Germany). The thousand-grain weight (TGRW) of the cleaned wheat was 42.78 g, and its specific weight is 1.415 g cm⁻³. The dimensions of 100 wheat grains after cleaning compared to those reported in the literature are shown in table 1.

The silo experiments required that two samples of wheat (i.e., samples with and without markers) could be differentiated from each other. Therefore, one sample was dyed with red food coloring (Cochineal Red A, E 124; Firma Wusita, Sitzendorf, Germany). As a result, the moisture content increased from 13.3% to 15.2%, as measured by a moisture meter.

MARKERS

For our experiments, we used a marker described by Beplate-Haarstrich (2008) with a density of 1.378 g cm⁻³ (fig. 1). The 3% density difference between the markers and the wheat can be expected to be negligible when compared to other segregation mechanisms (Gutiérrez et al., 2004; Schröter et al., 2006).

This marker is made of epoxy resin (R&G Faserverbundwerkstoffe GmbH, Waldenbuch, Germany). The correct density of the marker was ensured by the inser-



Figure 1. Comparison of simulated RFID markers with wheat grains used in these experiments.

tion of a 1.5 mm steel ball (Isometall, Pleidelheim, Germany). The insertion of the steel ball also had another purpose: it led to an increased absorption of x-rays in the CT imaging, making the marker more visible. This was important in the determination of the position of the markers in the second series of experiments using a shaker. The markers were produced using silicon forms according to Beplate-Haarstrich (2008). Two markers are shown in figure 1, showing the different surfaces (flattened and rounded). Each marker had a length of 6 mm, height of 3 mm, and width of 4 mm.

EXPERIMENT 1: SEGREGATION IN SILO DISCHARGE

Experimental Setup

The aim of the first experiment was to test if the ratio of two batches of wheat was well represented by the concentration of markers, which were added to only one batch, measured in samples taken from the outflow of a silo. The silo consisted of a slot-bottomed bin with a rectangular base (internal width of 476 mm, depth of 200 mm, and height of 2000 mm). The outlet was 150 mm wide and 200 mm deep (fig. 2). Core flow was the desired flow profile in this experiment. According to the method of Jenike (Schulze, 2006; Stieß, 2009), core flow requires an angle larger than 48°. In our design, we increased the angle even further to 70°.

Two differently colored samples wheat (a dyed sample without markers, and an undyed sample with markers) were poured into the silo in two layers (fig. 2). Three different concentrations of markers were used: 1 marker, 10 markers, and 50 markers per kg wheat. Each individual sample of wheat (undyed and dyed) weighed 30 kg.

A V-shaped (free-fall) mixer with a volume of roughly 20 L (made by the Section for Agricultural Engineering at the University of Göttingen) was used to produce an evenly distributed wheat-marker mixture. The design of the V-shaped mixer is described by Harnby et al. (1997). It consists of a hollow form made from two tubes at an angle of 60°. The outlet is situated at the connection point of the two tubes (fig. 2). An optimal mixing time of 1 min 20 s was determined according to Stieß (2009).

Experimental Method

The lower layer poured into the silo was 30 kg red-dyed winter wheat without markers. On top of this layer, 30 kg of undyed wheat, evenly mixed with a predefined number

Table 1. Dimensions of 100 wheat grains of cultivar *Kranich* compared with values given in the literature.

	Minimum-Maximum Dimensions (mm)			
	This Study	Tscheuschner (1996)	Kruse and Hackländer (2008)	Nelson (2001)
Thickness	2.50-4.10	1.50-3.80	2.40-3.20	2.40-2.90
Width	2.70-4.50	1.60-4.00	2.80-3.60	2.60-3.40
Length	5.60-7.40	4.20-8.60	5.50-6.50	5.60-6.40

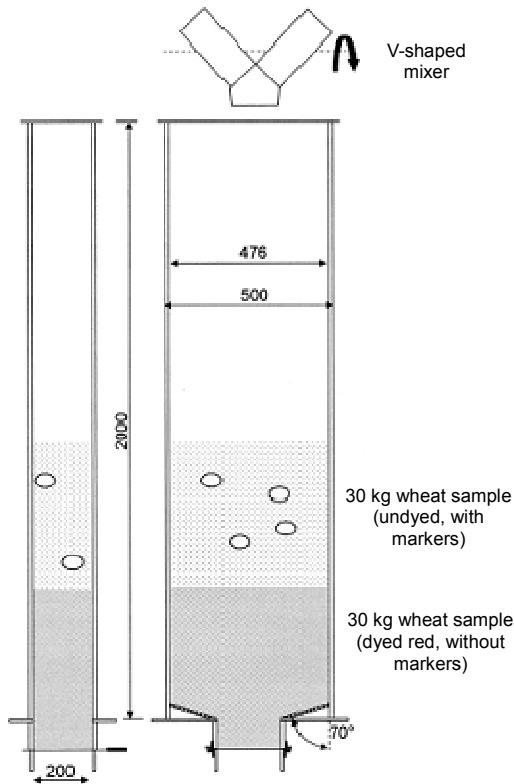


Figure 2. Experimental setup used to investigate segregation during silo discharge (measurements are in mm).

of markers, were poured. Due to the construction of the V-shaped mixer, the maximum amount of wheat that could be mixed at a time was 10 kg. Therefore, filling the silo with the wheat containing markers had to be done in three steps.

Three sets of experiments with different marker concentrations (1, 10, and 50 markers kg^{-1} wheat) were performed; each of these experiments was repeated three times. The total content of the experimental silo (2×30 kg wheat and markers) was emptied into six subsamples. This division was done manually, as emptying of the silo through the chosen outlet size took only a few seconds. For this reason, the weight of the six subsamples varied from 6 to 16 kg.

The number of markers and the amount of undyed wheat in each of the six subsamples was determined manually. First, the markers in the sample were sorted out using a bar magnet, making use of the embedded steel balls. From the counted number of markers and the known marker density, the “estimated mass” of undyed wheat was computed. The “actual mass” of undyed wheat was determined by taking a representative sample according to the method of Danzer et al. (2001). Then the numbers of undyed and total grains were counted manually. This ratio times the total mass of the subsample gave the “actual mass.”

EXPERIMENT 2: SEGREGATION DURING SHAKING

Vertical shaking is a standard method to fluidize granular samples and allow internal re-orientation and segregation. It can also be expected to mimic the excitation that a grain sample experiences during road transport. In this study, the shaking experiments were performed in two steps. First, a sample containing markers was mounted on

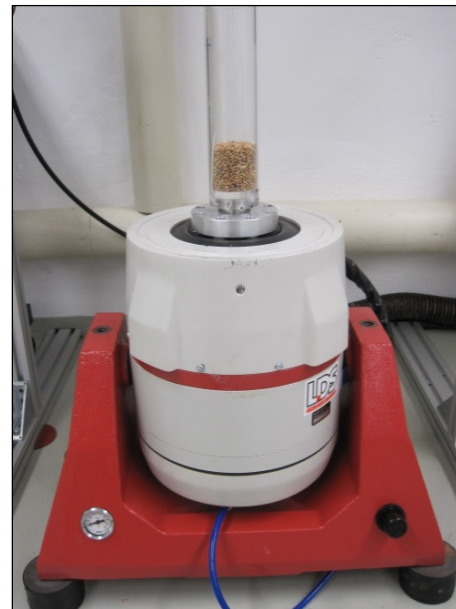


Figure 3. Plexiglass container used for segregation during shaking experiment mounted on top of the electromagnetic shaker.

an electromagnetic shaker, as depicted in figure 3. After shaking, the sample was inserted into an x-ray tomography setup, where the positions of all the markers were determined.

All samples were prepared in a plexiglass container of 50 mm inner width and 200 mm height. The sample consisted of 85 g winter wheat and 85 markers and filled the container to a height of 50 mm. To provide reproducible starting conditions, the sequence of filling was 42.5 g winter wheat, 85 markers, and again 42.5 g winter wheat. In preliminary tests, we found that this starting configuration resulted in a rapid distribution of the markers throughout the container during shaking. The low concentration of 85 markers in 1,987 wheat grains (calculated from the TGRW) ensured that the markers collided mainly with wheat grains during shaking and not with each other.

The filled container was screwed with a custom-made adapter onto an electromagnetic shaker (LDS V555, Ling Dynamics Systems, Herefordshire, U.K.) (fig. 3). It was then shaken using sinusoidal vibration with a given amplitude (A) and frequency (f). The maximum acceleration (a) during shaking is given by:

$$a = 4\pi^2 f^2 A \quad (1)$$

and is measured in units of g , the acceleration due to gravity. In our experiments, we kept the amplitude fixed and varied only the frequency. For each value of f , the experiment was repeated four times.

At the end of the vertical shaking, the container was placed inside a commercial CT scanner (Nanotom, GE Sensing and Inspection Technologies, Wunstorf, Germany), as shown in figure 4. To ensure optimal contrast, the samples were imaged using a voltage of 70 kV and a current of 90 μA . During each measurement, radiograms were taken while the container was rotating 360°. These radiograms were then combined to form a single three-dimensional CT

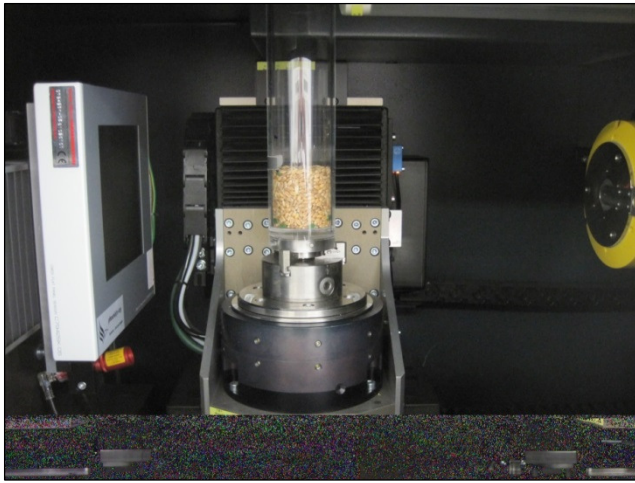


Figure 4. Setup used for x-ray tomography. The container is mounted on the rotation table, on the left is the Hamamatsu detector, and on the right is the yellow x-ray tube.

image with $512 \times 512 \times 512$ voxels (a voxel is the three-dimensional equivalent of a pixel) using the system's software. Figure 5b demonstrates the amount of information obtainable from such tomograms.

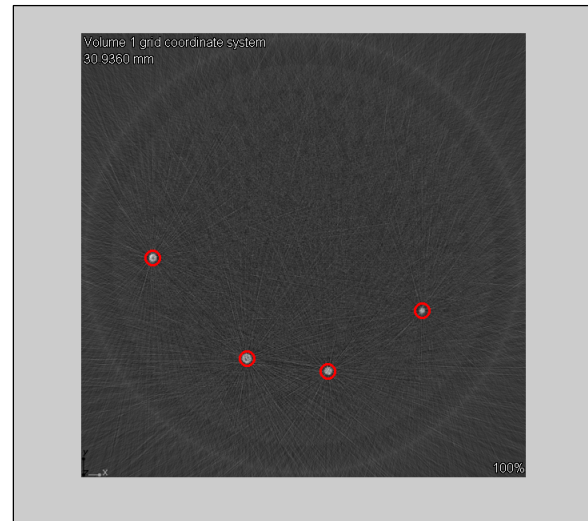
The resolution of the measurements was $121 \times 121 \times 121 \mu\text{m}$ per voxel. This resolution allowed us to image the whole sample from the bottom of the container to the grains lying at the top of the sample. The coordinates of each of the 85 markers were determined using binarization with ImageJ software and the Object Counter 3D plugin (Schneider et al., 2012). The embedded steel balls acted as representatives for each marker, and the epoxy resin capsule ensured that individual steel balls could be recognized separately even when two markers were in direct contact. The coordinates of the steel balls formed the basis for the evaluation of this experiment, as discussed in the Results section.

RESULTS

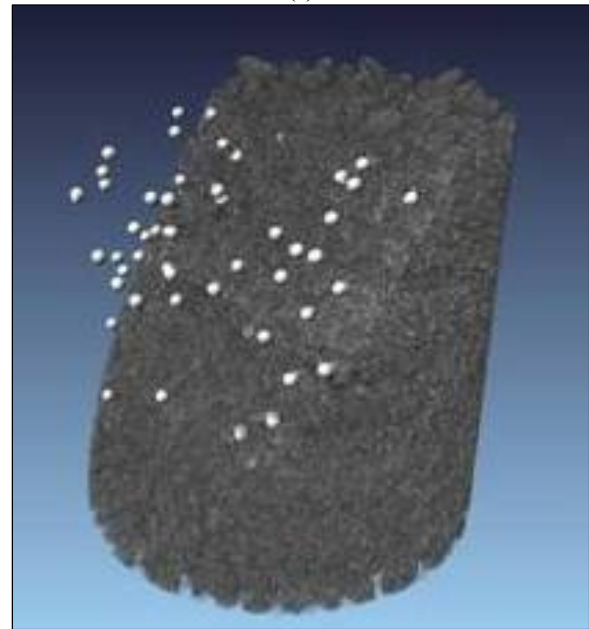
EXPERIMENT 1: SEGREGATION IN SILO DISCHARGE

Figure 6 shows the relationship between the true mass of the undyed wheat sample (sample A) discharged from the silo and its estimated mass calculated using the markers. For a perfect measurement, these two masses would be the same, and all data points would lie on the graph's bisector line. In reality, there will be deviations, which can stem from two causes. First, statistical fluctuations are due to the small marker concentration; even for $50 \text{ markers kg}^{-1}$, the number-based concentration of markers is only 0.21%. Second, if the system exhibits segregation of markers during discharge, then the estimated mass will deviate in both directions from the actual mass.

To distinguish between these two causes, we performed an analysis of the statistical deviations that can be expected from the low marker concentration. As outlined in the Appendix, the relative error of the measured mass (which is defined as the difference between the actual mass and the estimated mass divided by the actual mass) depends on



(a)



(b)

Figure 5. CT images of the marker-wheat mixtures after vertical shaking: (a) horizontal cross-section through the container (the four markers visible in this slice are marked with red circles), and (b) 3D surface image (the wheat grains in the upper left segment have been made transparent, allowing the markers to become visible).

both the marker concentration and the sample size. Two statistical concepts can be applied to measure the relative error for a given marker density. Frequentist statistics provide an expression for the average error that can be expected for a given sample size. Bayesian statistical analysis can answer the question: What is the minimum sample size that ensures that the relative error of the estimated mass will be smaller than $X\%$ with respect to a 95% confidence level?

Figure 7 shows the relative error of the estimated mass as a function of the actual mass and a marker density of $50 \text{ markers kg}^{-1}$. In particular, figure 7 shows that for a real mass of at least 1.5 kg (or a sample including at least 75 markers), the average relative error is smaller than 10%. However, if 95% confidence is required for the relative

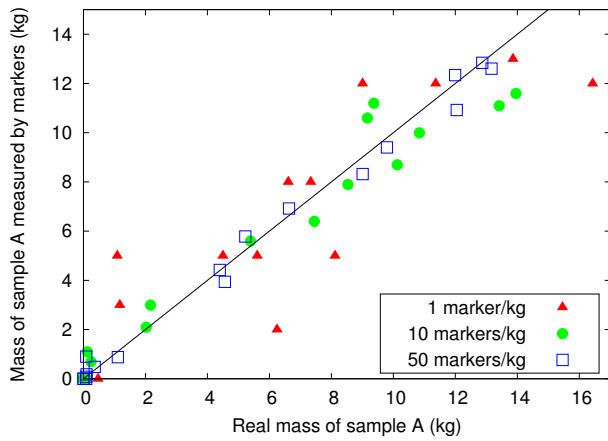


Figure 6. Relationship between the estimated mass of undyed sample (from the measured number of markers) and its true mass (from hand-counting the ratio of undyed to total grains). The bisector line corresponds to an ideal, error-free measurement. The deviation from this ideal decreases with increasing marker concentration. Each data point corresponds to one wheat sample.

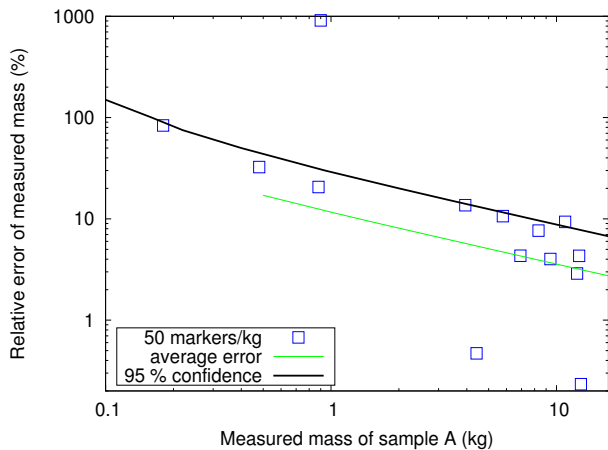


Figure 7. Discharge from the silo did not lead to segregation of the markers. This follows from the fact that the relative error of the mass of wheat determined from the number of markers is well described by a statistical analysis that assumes only random fluctuations. This is true for both the expected average error (lower line, based on frequentist statistics) and the 95% confidence level of the relative error (upper line, based on Bayesian analysis).

error of the measured mass to be smaller than 10%, then at least 7.74 kg of wheat (i.e., a sample with 387 markers) are needed for analysis. The average relative error and the 95% confidence boundary both provide a good description of our data. Therefore, we can conclude that there was no measurable additional segregation during our silo discharge experiment.

EXPERIMENT 2: SEGREGATION DURING SHAKING

Hinsch et al. (1993) and Barchi et al. (2002) studied the mechanical vibrations that crops are exposed to during transport on trucks. They observed strong amplitudes in the range 3.5 to 46 Hz, with details depending on the suspension system of the truck, the position of the sensor, and apparently also the geographical area of the measurement. To establish reproducibility, we performed our experiments

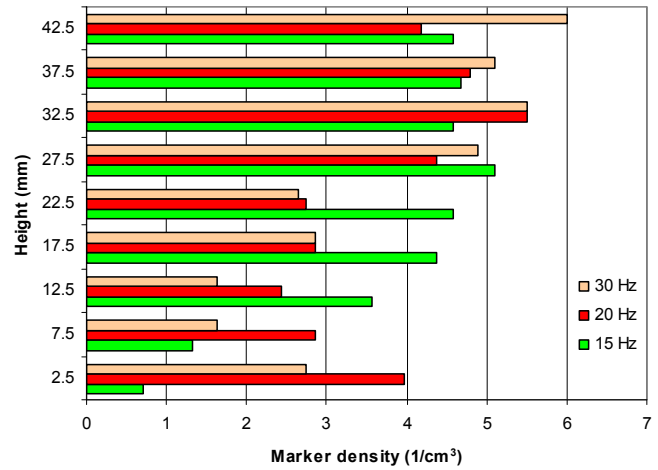


Figure 8. Vertical density distribution showing that marker density increased with height in the container. The horizontal segments are 5 mm high, and each bar represents the average of four experiments.

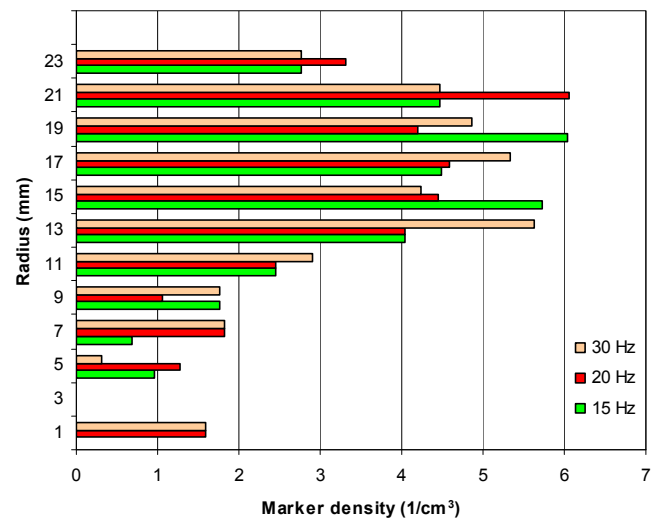


Figure 9. Radial density distribution showing that markers accumulated closer to the container boundaries. Each bar represents a cylinder with 2 mm wall thickness and an average of four experiments.

at an amplitude of 2.5 mm and frequencies of 15, 20, and 30 Hz, which correspond to accelerations of 2.3g, 4g, and 9g according to equation 1. Visual observation ensured thorough mixing of the samples by a stable convective roll inside the container. This flow pattern was driven by the frictional forces between the particles and the container walls (Schröter et al., 2006). Each experimental sample was shaken for 10 min after its preparation, as described in the Materials and Methods section.

For analysis, we first computed the marker concentration per cubic centimeter from the marker positions determined by tomography. Then the dependency of this density on both the height in the container and the distance from the container's central vertical axis was measured. The results of the vertical density distribution are shown in figure 8, and those of the radial distribution are shown in figure 9. If the shaking had led to a thorough admixing of the markers and wheat, then there would have been a uniform density of 3.5 markers cm^{-3} throughout the sample.

Figure 8 shows that the average density in the upper part

of the container is higher than in the lower part. This is most apparent at 15 Hz but is also apparent at the other two frequencies. As the markers have a slightly greater volume than a wheat grain (fig. 1), this result can be designated as a mild Brazil nut effect (BNE), whereby larger particles tend to collect on the surface of a granular sample. This effect seems to decrease with increasing frequency.

At a shaking frequency of 20 Hz, a greater proportion of markers was found at the bottom of the container (height = 2.5 mm). This became obvious during the experiments: during shaking, some markers got stuck in the corner between the container's sidewall and its base. These markers had aligned their flat surface (fig. 1) with the container bottom, and no upward pointing forces could be exerted by the other grains.

The radial distribution shown in figure 9 shows an even more obvious degree of segregation. The majority of the markers were found lying in the outer region of the cylinder. This result was independent of the frequency.

DISCUSSION

A surprising result of the Bayesian analysis, described in the Appendix, is that the 95% confidence level for a certain error margin does depend only on the number of markers found, i.e., the product of marker concentration and sample mass. In figure 10, all experiments, using the three different marker concentrations, are plotted against the 95% confidence level of the Bayesian analysis. As this curve also provides a fair description of the relative errors, we again conclude that segregation was negligible in our setup.

The Bayesian analysis is also interesting from a different perspective. For many applications, one would care less for correct identification of samples in the single-digit kilogram range than for much larger samples. Figure 10 and the equations in the Appendix provide a guideline for choosing the necessary marker concentration under the assumption that no noticeable segregation occurs during the considered transport and handling chain.

The segregation observed in the vertically shaken sam-

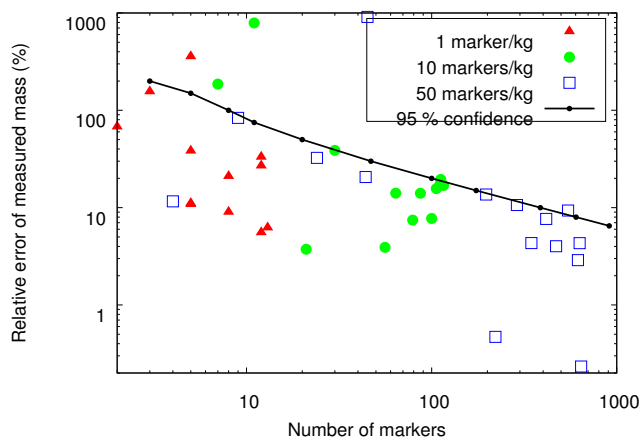


Figure 10. Relative error of the mass of marked wheat is determined solely by the number of markers found in the sample. This result, which is further explained in the Appendix, again demonstrates that the silo discharge did not induce segregation of the markers.

ple resulted in an increased concentration of markers in the upper and outer regions of the container. A comparison of this density distribution with the typical segregation mechanisms that occur in vertically shaken bulk materials shows that this segregation was most probably caused by convection rolls (Schröter et al., 2006). These convection rolls are a consequence of the friction between the particles and the container walls, which leads to a downward movement of the grains next to the sidewalls. This flux is then compensated by an upward movement in the center of the container. Larger particles experience more difficulties entering the outer downward stream and therefore tend to collect in the upper parts of the container near the surface (i.e., the BNE). In our experiments, this effect can be considered to be comparatively mild, given the experimental conditions (Schröter et al., 2006).

As discussed above, a second effect of the shaking was the collection of markers at the bottom of the container at 20 and 30 Hz. This entrapment did not occur at lower frequencies, and we assume that the convection roll did not enter this region. This observation is not segregation in the classical sense but a geometrical “entrapment” due to the markers having a flattened side. However, both of these effects can be reduced by a better design of the markers.

CONCLUSIONS

Our experiments on shaken wheat showed that the artificial RFID marker used in this study will exhibit some amount of segregation during transport. The mechanism increasing the marker density in the upper part of the vessel was sidewall-driven convection rolls. However, rounding the flattened upper surface and a small reduction in the volume of the marker can be expected to reduce this segregation considerably. In the discharge from our test silo, the weight fraction of the marked sample was well represented by the markers found in the outflow. The remaining errors can be solely explained by statistical fluctuations, which demonstrate the absence of segregation during outflow. Additionally, our statistical analysis of this experiment showed that the confidence level of the measurement depends only the actual number of markers in the sample, and not the marker concentration and sample mass separately. In summary, our results demonstrate that the marking of individual batches of wheat with artificial markers is a feasible option.

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APPENDIX

This Appendix provides the mathematical background for estimation of the sample mass from the number of markers and the sample density, as well as determination of the relative error, including its average value and its confidence intervals. All examples are calculated for a TGRW of 42.06 g, which corresponds to 23,775 grains kg^{-1} .

FREQUENTIST STATISTICS

We start by considering a sample of N wheat grains and a marker density of p . How many markers can we expect to find? The answer is given by a binomial distribution. The probability of finding k markers is equal to:

$$B(k, N, p) = \binom{N}{k} p^k (1-p)^{N-k}$$

with the binomial coefficient:

$$\binom{N}{k} = \frac{N!}{k!(N-k)!}$$

A binomial distribution for 1.6 kg of wheat and a marker density of 50 grains per kg is presented as graph A in figure A1, while graph B shows the binomial distributions for an entire range of sample weights with color-coded probabilities. For such binomially distributed markers, we know that the average number of markers per sample is pN , and the standard deviation is $\sqrt{p(1-p)N}$. We can calculate the average absolute error as:

$$\sum_{k=0}^N |Np - k| B(k, N, p)$$

which was done for the curves presented in figure 7 by using a marker density of $p = 50/27,375$ and sample sizes N , which correspond to masses between 0.5 and 16.0 kg.

BAYESIAN STATISTICS

For many practical applications, a related but slightly different problem must be investigated: given the number of markers (k) and the marker density (p), what is the number of grains (N) and consequently the mass of the sample? Bayesian inference (Gelman et al., 1995; Eddy, 2004) is applied to answer this question. In the context of Bayesian statistics, samples with different numbers of markers and with different sizes are analyzed. The above considered distribution of the number of markers (k) in a sample of size N is the conditional distribution of markers given a sample of size N and is denoted $P(k|N)$. In order to infer the sample size from the number of markers, we need an expression for the conditional distribution $P(N|k)$, i.e., the

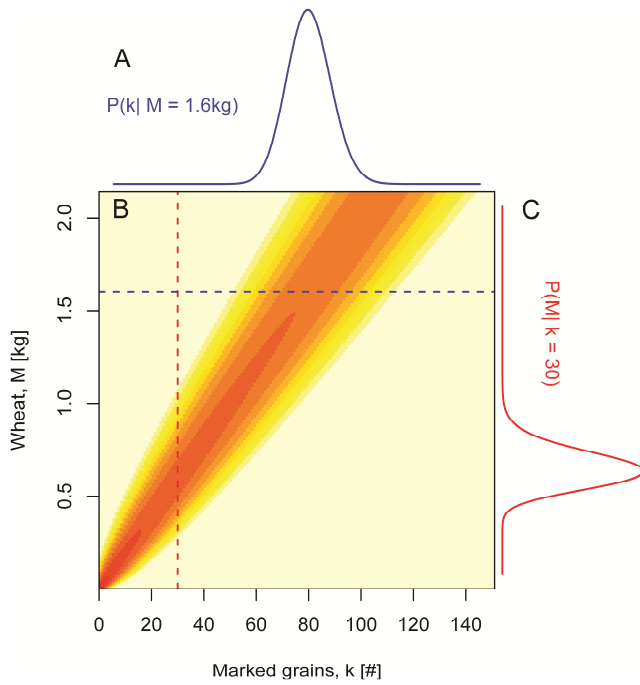


Figure A1. Modeled probability distributions of sample masses and number of markers. Graph A shows the probability distribution of markers for a sample mass of 1.6 kg. Graph B shows the probability distributions of markers for sample masses ranging from 0 to 2.14 kg. The color coding ranges from negligible (beige), small (yellow), intermediate (orange) to large (red) probabilities. Graph C shows the probability distribution of the sample mass for a fixed number ($k = 30$) of markers. All graphs assume $p = 50/27,375$.

distribution of sample sizes N for k markers found.

Following Bayes theorem, this conditional distribution can be derived by $P(N|k) = P(k|N)P(N)/P(k)$. The conditional distribution $P(k|N)$ is known: $P(k|N) = B(k, N, p)$.

The probability of the sample size N (the so-called prior) is assumed to be constant, $P(N) = 1/N_{\max}$, and the probability of finding k markers is:

$$\begin{aligned} P(k) &= \sum_{N'} P(k|N')P(N') \\ &= 1/N_{\max} \sum_{N'} P(k|N') = 1/(pN_{\max}) \end{aligned}$$

which leads to the desired distribution:

$$P(N|k) = pB(k, N, p)$$

An example of the corresponding sample mass distribution for $k = 30$ markers is presented in graph C of figure A1. The mean value of this distribution, i.e., the average size of a sample with k markers, is:

$$\mu[N(k)] = -1 + (k+1)/p$$

which is the Bayesian estimator of the sample size:

$$\hat{N}_B(k) = \mu[N(k)]$$

The standard deviation of $\hat{N}_B(k)$ is:

$$\sigma = \sqrt{k+1 - (k+1)p} / p$$

Note that for small values of k and low marker densities (p), the estimated sample sizes $\hat{N}(k)$ are very different from those gained by the frequentist statistics:

$$\hat{N}_F(k) = k / p$$

For example, if $k = 0$ markers are found, the estimated sample size is:

$$\hat{N}_B(k) = -1 + 1/p$$

which leads to an estimated sample weight of almost 1 kg for a marker density of 1 marker kg^{-1} . This result reflects that small samples (of just a few kilograms) are likely not to contain a single marker.

CONFIDENCE LEVEL

How reliable is our result of the estimated sample size? We quantify the reliability by the relative error:

$$\bar{E}_{rel} = |N_{true} - N_{measured}| / N_{true}$$

If k markers are found, our estimated number of grains is:

$$N_{measured} = \hat{N}_B(k)$$

As the true number of grains is unknown, we consider its distribution $P(N|k)$. The average relative error is then given by:

$$\bar{E}_{rel}(k) = \sum_N P(N|k) |N - \hat{N}_B(k)| / N$$

An example of the average relative error is given by the lower line in figure 7, but it can be higher for a specific sample. Therefore, to have high confidence, it is desirable for $E_{rel}(k)$ to be smaller than a certain threshold of $X\%$ (e.g., $X = 10$, $X = 15$, or $X = 20$). A relative error of $X\%$ or less requires true sample sizes N from N_l to N_u :

$$N_l = (1 + X/100)^{-1} \hat{N}_B(k)$$

$$N_u = (1 - X/100)^{-1} \hat{N}_B(k)$$

The confidence level for a relative error smaller than $X\%$ is then:

$$\sum_{N=N_l}^{N_u} P(N|k)$$

which increases with the number of markers (k). The same formalism can be used to derive 95% confidence levels for the relative error. For each number of markers (k), the percentage X (and its corresponding quantities N_l and N_u) is determined that fulfills:

$$\sum_{N=N_l}^{N_u} P(N|k) = 0.95$$

An example for such a 95% confidence level X is presented by the upper line in figure 7.

Finally, for the three marker densities used in this article, we have numerically determined the minimum number of markers (k_{\min}) required to reach a confidence level of 95% for relative errors of less than 10%, 15%, and 20%. The results, which are summarized in table A1, are essentially equal for the different marker densities (p) that we consider here. Therefore, if 390 (or 100) markers are found in the sample, then the relative error of the estimated sample size is smaller than 10% (or 20%) with respect to a confidence level of 95%.

Table A1. Minimum number of markers needed for a relative error of less than 10%, 15%, or 20% with respect to a confidence level of 95%.

Marker Density p (markers kg ⁻¹)	Maximum Relative Error (%)	Minimum Number of Markers Needed (k_{\min})	Minimum Weight of Sample (kg)
1	10	388	389.00
10	10	388	38.90
50	10	387	7.76
1	15	175	176.00
10	15	175	17.60
50	15	174	3.50
1	20	100	101.00
10	20	100	10.10
50	20	100	2.02