Research Article

Dominika Barbara Matuszek*

Fluorescence in the assessment of the share of a key component in the mixing of feed

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Abstract: This paper presents the results of the mixing of a multicomponent feed for cattle. Three types of mixtures with different proportions of individual components and granulometric composition were selected. After the mixing process, the fraction of the key component (tracer) was determined. Tracer consisted of crushed grains of yellow maize, which was wet treated with a 0.01% solution of Rhodamine B. A tracer with two different average particle sizes d_1 = 2.0 mm and d_2 = 1.25 mm was introduced into the mixture. Then, the sample was illuminated with UV light, and the content of the tracer in the sample was evaluated using the computer image analysis. In addition, the tracer was separated to determine its fraction using a laboratory scale. From the obtained results, the high reliability of the fluorescence optical method for the evaluation of the homogeneity of granular multicomponent mixtures was proved. It was also observed that slightly better results were obtained for a tracer with a larger average particle size (d = 2.0 mm), although the comparative analysis did not indicate a significant statistical difference in the results in each series of tests.

Keywords: fluorescence, tracer, multicomponent granular mixtures, mixing of granular components

1 Introduction

The theoretical basis about the mixing of granular materials has been described since the 1940s [1–4]. This process takes place through a chaotic and random movement of the grains; however, many other behavioral conditions of grains are observed at the microscale level [4,5]. The behavior of the granular particles depends on their characteristics such as size, density, humidity, shape, and angle of repose, on the morphology of surface that has contact with the grains, and on the parameters of the mixing process itself [2,4–9]. The appropriate degree of mixing of granular components is an important parameter of the quality of the final product (e.g. in the context of nutrition) [10,11]. Therefore, it seems to be important to continuously develop the tools aimed at a better understanding of this complex and multifaceted process.

Homogeneity of the grain mixtures can be determined by degrees and indexes of mixing based on the content of a given component in samples [4,12,13]. In studies on the homogeneity of the grain mixtures, the correct sampling and analysis are important. The samples taken should be representative, and their quantity and size should be appropriate to the volume of the mixed material. The problem of sampling has been studied by many scientists [14–17]. The size of an individual sample also depends on the methodology adopted for determining the homogeneity of the mixture. For feeds, the reference method was based on the content of the active substance (e.g. tyrosine) [18]. However, research in this area, carried out for many years, has allowed the development and implementation of new research tools based on tracking the behavior of various types of tracers [19]. One of them is the method of assessing the homogeneity of feed, especially industrial feed, based on Mictracer® or Microgrids content [12,20–22]. There is also a continuous development of methods based on tracking individual particles using computer image analysis. Most such solutions were tested in the mixing of binary systems, where whole grains of different origin (biological materials, plastic materials, glass and steel balls, or chemical materials such as aluminum oxide balls and others) were mixed. Additionally, these ingredients differed in color [23–28]. In the methods based on image analysis, a component whose fraction was estimated was often colored. In the research conducted by the author of this paper,

* **Corresponding author: Dominika Barbara Matuszek,** Department of Biosystems Engineering, Faculty of Production Engineering and Logistics, Opole University of Technology, ul. Mikolajczyka 5, PL-45-271, Opole, Poland, e-mail: d.matuszek@po.edu.pl

substances featuring fluorescence under the influence of ultraviolet radiation (Tinopal, Rhodamine B, Uranine, Eosine) were used for dyeing [29–32].

This paper presents one of the stages of research conducted by the author for several years. So far, a series of tests have been performed mainly with the use of granular multicomponent mixtures containing whole grains [29,31,32]. Recent studies, including those presented below, concern the mixing of components after fineness [29]. In this aspect, a research problem has been addressed to verify the possibility of using fluorescence to assess the homogeneity of multicomponent mixtures subjected to the grinding process. Due to the high degree of fineness of the tested mixtures (M = 0.54–0.58 mm), it was necessary to use a tracer with a different average of particle size than before, namely 1.25 and 2.00 mm. This resulted from a certain assumption, namely, the desire to develop a method that would allow to determine the quality of the mixtures based on the content of a key component having the characteristics of mixed material, due to which the implemented experiments have a more practical character.

2 Methods

The studies were carried out for three multicomponent feeds for cattle obtained from the feed factory. The basic characteristics of these mixtures are listed in Table 1. Mixes no. 1 and 3 consisted of eight ingredients, while mix no. 2 – seven. The degree of fineness of mixtures (based on PN-R-64798: 2009-Feed. Determination of comminution) was in the range M = 0.54–0.58 mm. The mixing process was carried out using a laboratory flow mixer, which is built of two identical tanks (height of the cylindrical part – 200 mm, internal diameter 150 mm, and internal diameter of the outlet – 30 mm) placed in the construction (one above the other) enabling easy changes of their places. Mixing was carried out during 10 flows. It was proved that 10 flows in this type of mixer are enough to get the random state of mixture [33]. After the set time, 10 samples (N = 10) were taken from the entire volume of the mixed bed. This was possible by the modular design of the receiving tank (10 rings) and that's why 10 samples. The information about the flow mixer was presented in detail in earlier works [33,34].

The key component (tracer), i.e. yellow maize, was ground with different mesh sizes in a cereal mill. In this way, maize with two average particle sizes d_1 = 2.00 mm and d_2 = 1.25 mm was obtained. The component prepared in this way was then wet treated with a 0.01% solution of Rhodamine B ($C_{28}H_{31}ClN_2O_3$, excitation area 553 nm). Prior to mixing, 100 g of tracer (10% of the whole mixture) was introduced to the mixer in the same way. The remaining part of the mixed bed (90%, 900 g) was the compound feed. After the mixing process was completed, 10 g of single sample was taken and placed in a chamber equipped with two ultraviolet lamps. There was a digital camera in the upper part of the test station. In this way, images with a resolution of 1,600 × 1,200 pixels were obtained. The images were then analyzed using Patan software; this program was based on the RGB-256 scale. In the area of the acquired images, the colors responsible for the key component and background were determined. Exactly three classes were determined; 1-tracer, and 2 and 3-background. The designation of the two classes defining the background was due to the multicolor of the sample, due to which the obtained results were more reliable. Image post-processing consisted of assigning a color suitable for a given class (1, 2 or 3, first stage) to each of the pixels of the image and then determining the surface occupied by this class (second stage). The error in the first step of procedure equals to $256^{-3} = 5.9 \times 10^{-8}$; so, it is negligible, and during the second step, it equals 5.2×10^{-8}; so, it can be omitted in further calculations too. The information about the percentage share of individual classes in the analyzed area was obtained. In the following, the results of 1 class (tracer) participation were used.

Additionally, the fraction of the key component was evaluated by the control method, called as method 2 in

Table 1: Composition and degree of fineness of the analyzed mixtures

Types of components	Percentage of components		
	Mixture 1	Mixture 2	Mixture 3
Fodder chalk	1.5	2	8
Barley	—	—	2
Maize	36.5	30	10
Triticale	15	—	—
Soya meal	9	5	60
Rape meal	27	35	7
Dry corn decoction	10	25	—
Sodium chloride	0.5	0.5	3
Phosphate	—	—	3
Premix	0.5	2.5	5
Number of components	8	7	8
Fineness degree,[a] M (mm)	0.54	0.57	0.58

[a] Calculated based on PN-R-64798:2009.

this paper. This method was based on the manual separation of fluorescent maize from samples subjected to image analysis and then weighing on a laboratory balance with an accuracy of ±0.01 g. The separation of grains, for example, by sieves is, or rather was, the commonly used method (most important and known) to assess the content and then the parameters of chosen components of the mixture. In this way, the content of the introduced tracer (ground maize grains with average particle size d_1 = 2.00 mm and d_2 = 1.25 mm) was analyzed by two methods:

(1) Fluorescent method based on computer image analysis (Figure 1).
(2) Weighing method.

Three series of tests were performed for each tracer (maize d_1 = 2.00 mm and d_2 = 1.25 mm), obtaining 10 samples from each test; each series test was repeated three times.

These two methods were necessary for verification purposes. Based on the results obtained, the basic statistical parameters, the magnitude of the difference between the results obtained by the two methods (as a module of $x - x_0/x$, where x is the share of tracer assess by method 1 and x_0 is the share of tracer assess by method 2), and the coefficient of variation (as 100 × standard deviation/mean) were calculated. The percentage of the coefficient of variation, in this case, was used to assess the homogeneity of the multicomponent feed. Additionally, a statistical comparative analysis was performed based on parametric and nonparametric comparative tests depending on the normality of variable distribution (Shapiro–Wilk test verification) and homogeneity of variance (Levene test verification).

Table 2: Results of mixing of compound feeds for cattle using a tracer with an average particle size d_1 = 2.00 mm

Series of tests	Tracer share (%)		Difference[c] (%)
	Method 1[a]	Method 2[b]	
Mixture 1			
1	9.36 ± 2.39	9.22 ± 2.14	4.35 ± 2.62
2	9.80 ± 1.03	9.66 ± 1.18	3.40 ± 2.0
3	9.42 ± 0.84	9.47 ± 0.87	2.78 ± 1.35
Mean, %	9.52 ± 1.62	9.45 ± 1.53	3.51 ± 2.19
CV, %	14.99 ± 7.49	14.85 ± 6.02	8.9 ± 4.45
Mixture 2			
1	9.08 ± 0.86	9.08 ± 0.93	2.99 ± 1.44
2	9.33 ± 1.17	9.40 ± 1.16	3.13 ± 1.16
3	9.10 ± 1.14	9.05 ± 1.29	3.55 ± 1.93
Mean, %	9.17 ± 1.09	9.17 ± 1.14	3.22 ± 1.60
CV, %	11.52 ± 1.44	11.88 ± 2.09	5.68 ± 4.27
Mixture 3			
1	8.93 ± 1.00	8.87 ± 0.93	3.08 ± 1.45
2	9.16 ± 1.23	9.01 ± 1.32	4.01 ± 2.55
3	9.01 ± 0.91	9.07 ± 1.00	2.37 ± 1.39
Mean, %	9.03 ± 1.08	8.98 ± 1.12	3.15 ± 2.02
CV, %	11.58 ± 1.41	12.02 ± 1.83	7.40 ± 0.70

[a]Arithmetic mean of the percentage of the tracer obtained by computer image analysis ± standard deviation. [b]Arithmetic mean of the percentage of the tracer obtained by weighting ± standard deviation. [c]Difference in results obtained by the two methods ± standard deviation.

The significance level α = 0.05 was adopted for calculations.

Ethical approval: The conducted research is not related to either human or animal use.

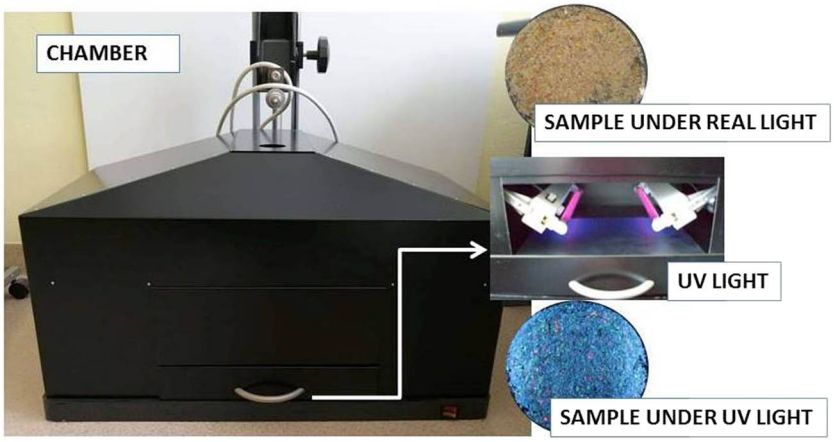

Figure 1: Schematic representation of the setup of the optical fluorescent method.

Table 3: Results of mixing of compound feed for cattle using a tracer with an average particle size of d_2 = 1.25 mm

Series of tests	Tracer share (%)		Difference[c] (%)
	Method 1[a]	Method 2[b]	
Mixture 1			
1	8.65 ± 1.17	8.79 ± 1.06	6.93 ± 4.01
2	9.07 ± 1.52	8.99 ± 1.30	7.53 ± 5.39
3	9.10 ± 0.90	9.30 ± 0.96	5.68 ± 3.95
Mean, %	8.94 ± 1.26	9.03 ± 1.15	6.71 ± 4.64
CV, %	13.38 ± 2.79	12.26 ± 1.70	10.68 ± 5.01
Mixture 2			
1	8.87 ± 1.07	8.73 ± 0.97	9.98 ± 10.77
2	8.82 ± 1.32	8.84 ± 1.06	8.06 ± 5.41
3	8.81 ± 0.94	8.72 ± 1.04	9.93 ± 2.85
Mean, %	8.83 ± 1.14	8.76 ± 1.04	9.32 ± 7.33
CV, %	12.58 ± 1.81	11.67 ± 0.41	14.49 ± 7.11
Mixture 3			
1	8.76 ± 0.99	8.84 ± 0.83	11.29 ± 5.93
2	9.73 ± 1.22	9.66 ± 1.00	9.25 ± 6.27
3	9.48 ± 1.28	9.59 ± 1.21	9.95 ± 6.74
Mean, %	9.32 ± 1.26	9.36 ± 1.11	10.16 ± 6.49
CV, %	12.43 ± 0.87	10.76 ± 1.35	16.32 ± 6.60

[a] Arithmetic mean of the percentage of the tracer obtained by computer image analysis ± standard deviation. [b] Arithmetic mean of the percentage of the tracer obtained by weighting ± standard deviation. [c] Difference in results obtained by the two methods ± standard deviation.

3 Results and discussion

The results of the share of tracer with two degrees of fineness obtained by the two methods are listed in Tables 2 and 3. The graphical interpretation of the tracer share obtained by the fluorescent method (marked as blue square) and weight method (marked as orange square) is presented in Figure 2 (for tracer 2.00 mm) and in Figure 3 (for tracer 1.25 mm), respectively. Figure 4 presents the value of difference obtained in relation to the control method for a tracer with diameters 2.00 and 1.25 mm and three mixtures (mix no. 1: blue square; mix no. 2: orange square; and mix no. 3: green square).

Based on the results listed in Tables 2 and 3 and their graphical interpretations in Figures 2 and 3, a small variability can be observed in the share of the tracer estimated by the two methods. The lack of significance of these differences was proved by statistical comparative analysis. This observation is similar for a series of tests using maize with a finer (d_2 = 1.25 mm) and a larger average of particle size (d_1 = 2.00 mm). However, it can be observed (Tables 2, 3 and Figure 4) that for the tracer with an average of particle size of d_1 = 2.00 mm, a lower

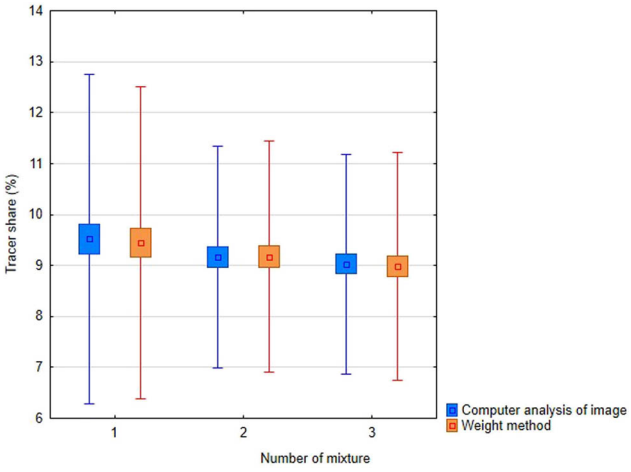

Figure 2: A box plot graph of the share of the tracer (maize d = 2.00 mm) obtained by two methods for the three mixtures analyzed (point: mean, box: mean ± standard error, whiskers: mean ± 2 × standard deviation).

level of difference in results (method 1) was obtained when compared with the control method (method 2). In addition, it seems that the deviation of the share of key components for mix no. 1 (mixing using tracer with 1.25 mm) is greater (9.52 ± 1.62% for method 1 and 9.45 ± 1.53% for method 2). The explanation of this is extremely difficult because of the course of the mixing process, and thus, the location of the tracer in a mixed bed depends on many factors such as the number and share of individual components or their features such as dimension, density, humidity, shape, and others. The lowest level of difference was 2.37 ± 1.39% and the highest was

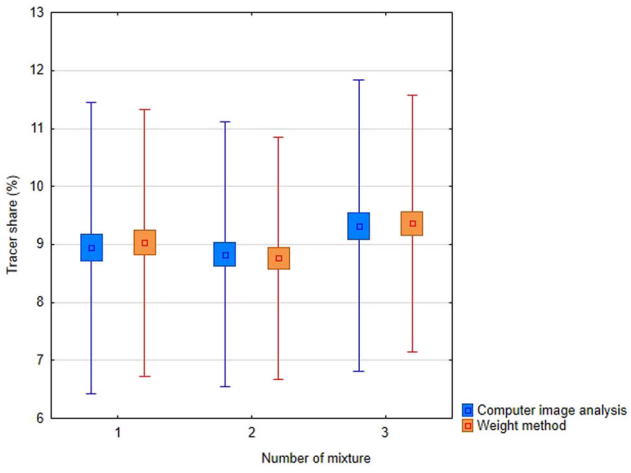

Figure 3: A box plot graph of the share of the tracer (maize d = 1.25 mm) obtained by two methods for the three mixtures analyzed (point: mean, box: mean ± standard error, whiskers: mean ± 2 × standard deviation).

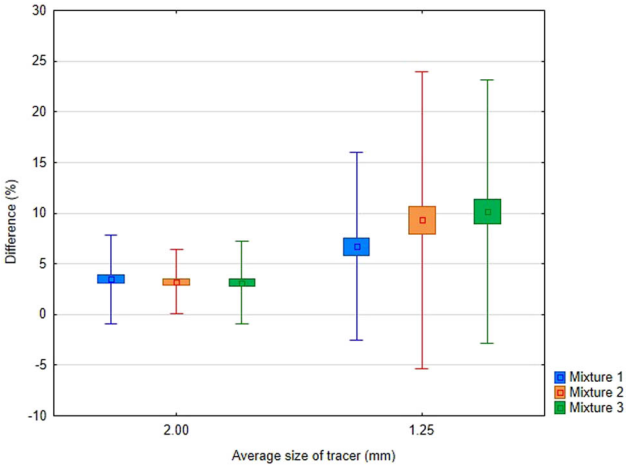

Figure 4: A box plot graph of the difference between the results obtained by the two methods for the average size of the tracer d_1 = 2.00 and d_2 = 1.25 mm (point: mean, box: mean ± standard error, whiskers: mean ± 2 × standard deviation).

4.35 ± 2.62%. The average range of the difference was 3.15–3.51%. For a series of tests involving maize with an average particle size of d_2 = 1.25 mm, the differences obtained were greater. The lowest value of this difference was 5.68 ± 3.95% and the highest was 11.29 ± 5.93%, with mean values ranging between 9.32 and 10.68. This difference is particularly noticeable in Figure 4. Similar observations were made in relation to the obtained results of the coefficient of variation. In mixing with fluorescent maize d_1 = 2.00 mm, the average difference amount is in the range of 5.68–8.90%. However, in tests with d_2 = 1.25 mm, the value was obtained in the range of 10.68–16.32%.

The significance of differences in the results obtained by the two methods was verified based on the relevant statistical tests. For this purpose, the Student's *t*-test and *U* Mann–Whitney's test for the significance level α = 0.05 were used. The non-parametric (*U* Mann–Whitney) test was used for one case where verification did not confirm the normal distribution of variables. In other cases, the parametric test was used. The results of the relevant tests are summarized in Table 4 and its graphical interpretation is shown in Figure 4.

The results of statistical comparative analysis (Table 4) indicate that there are no significant statistical differences between the results obtained by the two methods. Therefore, the previously observed larger deviations of the results compared to the control method for d_2 = 1.25 mm maize are not statistically significant. Therefore, it can be concluded that the use of the fluorescence phenomenon in the assessment of the content of the Rhodamine B-coated tracer with two different levels of fineness (d_1 = 2.00 mm and d_2 = 1.25 mm) gives reliable results.

Table 4: Results of statistical comparative analysis of the tracer's share obtained by the two methods

Mixture no.	Statistical test result	*p*
Tracer d = 2.00 mm		
1[a]	0.18	0.85628
2[a]	0.02	0.98343
3[a]	0.33	0.73940
Tracer d = 1.25 mm		
1[a]	−0.28	0.77791
2[a]	0.24	0.81212
3[b]	0.12	0.90163

[a]Parametric Student's *t*-test. Normal distribution of variables, homogeneity of variance. [b]Nonparametric *U* Mann–Whitney test. No normal distribution of variables, homogeneity of variance.

The multicomponent granular mixtures analyzed did not differ significantly in terms of the number of components or granulometric composition (Table 1). Therefore, it was not possible to determine whether these parameters affect the results obtained. To determine this correlation, it is necessary to have more results, which is a further stage of the tests conducted by the author.

The presented method used the computer image analysis and the phenomenon of fluorescence. The combination of these two elements allowed for a quick assessment of the tracer's share eliminating possible distortions in the analysis. These disturbances result from the properties of loose materials, such as the ability to cover the tracer, which may cause the underestimation of its share. The excitation of the fluorescent maize for lighting eliminated this problem. Of course, the issue of sampling still remains. This is a disadvantage of the proposed solution. However, compared with online methods, it does not require more advanced equipment with quite complicated operation [35–38]. These types of methods also have limitations, such as the need to provide a window for observing mixed materials [39]. Author developed a simple tool, whose operation was improved by the use of fluorescence in the ultraviolet. Moreover, no similar tool for assessing the homogeneity of loose mixtures has yet been developed.

So far, the method has been tested only in laboratory conditions. However, it is worth noting that industrial feeds were mixed and the tracer was maize (normally used as feed component). Due to the properties of the

fluorescent substance Rhodamine B (chemical substance with irritant properties), the method has limits in application to industrial conditions. In addition, the fluorescent dye can be rinsed out of the tracer when liquid additives are added to the mixer, which is sometimes practiced in feed production. However, it is possible that the use of appropriate models will allow transferring of the results obtained in laboratory conditions on a larger scale.

4 Conclusions

- Lower values of the differences in the results obtained by the two methods were observed when maize grains with an average particle size d_1 = 2.00 mm were used. However, the results of statistical comparative analysis do not indicate significant differences between the results obtained by the two methods: (1) the method of computer image analysis and (2) weight method for a tracer with two levels of fineness (2.00 and 1.25 mm).
- The use of a fluorescent substance and its excitation to light under ultraviolet light allowed for accurate estimation of the tracer's share against the background of the multicomponent mixture.
- The method based on the assessment of the fluorescent-coated component (Rhodamine B) can be used to monitor the share of the tracer in samples of high fineness multicomponent granular mixtures M = 0.54–0.58 mm. Therefore, the proposed solution can be used to assess the homogeneity of such mixtures.

Conflict of interest: The author declares no conflicts of interest.

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