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THE USE OF THE FLUORESCENCE PHENOMENON TO EVALUATE THE CONTENT OF FEED INGREDIENTS

Summary

This paper presents the results of the evaluation of feed quality conducted on the basis of the content of a tracer coated with a fluorescent substance. The study used the example of compound feed mixture for laying hens. Feed ingredients: maize and kardi primed wet using fluorescent solutions. The substances, such as Tinopal, Uranine and Rhodamine B were excited to shining by ultraviolet radiation. Before mixing process started, two tracers coated with different fluorescent substances were introduced into the mixture. The acquired images of the samples were analysed using the Patan computer program and were the basis of the information about the percentage share of the specified key component. The tests were used to verify the premise that there is a possibility of simultaneous determination of several components in a mixture based on fluorescent method. The results confirmed this assumption.
***Key words**: feed, tracer, homogeneity, fluorescence*

WYKORZYSTANIE ZJAWISKA FLUORESCENCJI DO OCENY ZAWARTOŚCI SKŁADNIKÓW PASZ

Streszczenie

W pracy przedstawiono wyniki oceny jakości paszy w oparciu o zawartość składnika kluczowego pokrytego substancją fluorescencyjną. Badania przeprowadzono na przykładzie wieloskładnikowej mieszanki paszowej dla kur niosek. Składniki paszy: kukurydze i kardi zaprawiano na mokro rozworami fluorescencyjnymi. Zastosowane substancje: tinopal, uranina i rodamina B wzbudzano do świecenie promieniowaniem ultrafioletowym. Pozyskane obrazy próbek poddawano analizie w programie komputerowym Patan, na podstawie którego uzyskiwano informacje o procentowej zawartości wskazanego składnika kluczowego. Do mieszanki przed rozpoczęciem procesu mieszania wprowadzano dwa trasery pokryte różnymi substancjami fluorescencyjnymi. Testy miały posłużyć do weryfikacji założenia, że istnieje możliwość jednoczesnego oznaczenia zawartości kilku składników w jednej mieszance w oparciu o metodę fluorescencyjną. Uzyskane wyniki potwierdziły założenie.
***Słowa kluczowe**: pasza, traser, homogeniczność, fluorescencja*

1. Introduction

As of today, the issues of the quality and safety of feed production relate particularly to the raw materials' content of heavy metals, presence of pathogenic organisms and mycotoxins, alternative sources of nutrients in feeds, the use of pharmaceuticals (coccidiostats) or innovative tools for quick and precise assessment of mixture quality [1, 9, 14, 15].

The studies on the development of methods and tools to assess the feed homogeneity are still the subject of interest. This is due to, among many factors, the complexity of the granular and loose material blending processes as well as to the new possibilities offered by new technological advances [3, 13]. Among numerous available studies are those making use of innovative visual techniques [2, 5, 7, 10, 12, 16]. The assessment of feed homogeneity is particularly difficult in industrial conditions where multicomponent systems are blended (i.e. the number of components is larger than three). Few studies were published as of yet on this subject [4, 6, 8]. In such conditions, the assessment of mixture homogeneity consists in determination of the content of a key component which may be either a component of the original composition or a deliberate addition to the blended system. Tracking the share of all mixed components is time-consuming and often impossible, on the other hand, the estimation of homogeneity of the mixture based on the participation of the indicator may not reflect the exact quality of the mixture.

The authors have developed a new method of analysis of feed homogeneity using the phenomenon of fluorescence [10]. This paper describes the extension of this method, namely, the results of testing of the simultaneous use of several fluorescent tracers in one mixture to evaluate various characteristics. The tool that allows for the observation of any additional feature plays an important role in the characterization of the kinetics of the mixing process and the quality of the final product.

The aim of the study is to assess the usefulness of the fluorescence method for determining the share of selected components of feed in a given sample.

2. Material and method

The mixing process was carried out for components of fodder for laying hens. The share of individual components is shown in Table 1.

Prior to mixing, two indicators: maize and kardi coated with fluorescent substances were introduced into the mixture in an amount of 100 g (10%). Because of the use of the RGB model for determination of the share of individual components of the image, the used tracers had to be different in terms of colours emitted after excitation by UV rays. The tests carried out in six runs are shown in Table 2.

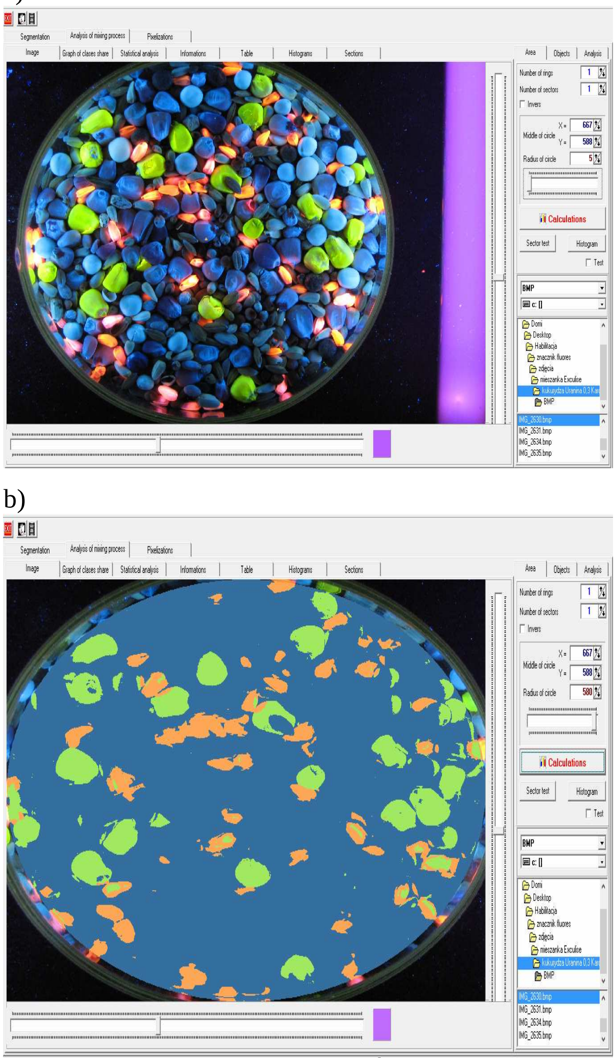
Table 1. The composition of the mixture for laying hens
Tab. 1. Skład mieszanki dla kur niosek

No.	Component	Share, %
1.	Maize	20
2.	Paddy Rice	13
3.	Kardi	10
4.	Pink maize	9
5.	Red sorghum	9
6.	Dari	9
7.	Green peas	5
8.	White peas	5
9.	Wheat	4
10.	Vetch	4
11.	Sunflower	4
12.	Field pea	4
13.	Millet	1.5
14.	Hemp	1.5
15.	Mung	1

Source: own work / Źródło: opracowanie własne

The applied types and concentrations of fluorescent materials are the result of previous tests carried out by the author [11]. Mixing was performed in a laboratory flow-through mixer. After completion of the mixing process (10 flows), the samples of each feed were taken from 10 different levels of the device. Mass of a single sample was 40 g. The samples were placed in a Petri dish measuring 120 mm x 20 mm and then in a chamber equipped with UV light. The acquired images of samples were analyzed using the computer application working based on the RGB model, marking the circular area and accordingly three objects for analysis: 1 - tracer 1, 2 - tracer 2, 3 - background. On this basis the information was obtained about the percentage share of the components. Figure 1 illustrates the exemplary image of a feed sample containing two essential components: maize coated with 0.3% solution of Uranine and kardi coated with 0.01% solution of Rhodamine B, in the ultraviolet light and after the analysis using the computer program. The method was verified by comparing the results with the percentage content of a given component obtained by weighing it on an electronic scales with an accuracy of 0.01g. A detailed description of the method for assessing the content of a key component using the fluorescent method was included in the other work of the author. It was assumed that the size of an error for results obtained using the two methods can be up to 5%. Furthermore, statistical comparative analysis was conducted for a series of tests meeting this assumption based on t-test (r = 2, k = 19) under the null hypothesis of the equality of data from the two methods. The statistical analysis was set at the level of significance $\alpha = 0.05$.

a)



b)

Source: own work / Źródło: opracowanie własne

Fig. 1. Image of the sample of mixture for laying hens: a) in the UV light, b) after the analysis using computer program
Rys. 1. Obraz próbki mieszanki dla kur niosek: a) w świetle UV, b) po analizie w programie komputerowym

Table 2. The combinations of conducted series of tests
Tab. 2. Kombinacje przeprowadzonych serii badań

Series number	Key component	Type and concentration of the fluorescent substance			
		Rhodamine B 0.01%	Tinopal 0.03%	Uranine 0.3%[1]	Uranine 0.5%[2]
1	Maize	X	-	-	-
	Kardi	-	X	-	-
2	Maize	X	-	-	-
	Kardi	-	-	-	X
3	Maize	-	X	-	-
	Kardi	X	-	-	-
4	Maize	-	X	-	-
	Kardi	-	-	-	X
5	Maize	-	-	X	-
	Kardi	X	-	-	-
6	Maize	-	-	X	-
	Kardi	-	X	-	-

[1] concentration for maize; [2] concentration for kardi

Source: own work / Źródło: opracowanie własne

3. Results and discussion

The results of tests conducted for each series (Table 2), their basic statistics (mean, standard deviation) and the results of comparative statistics (for series meeting the assumption $\delta x \leq 5\%$) are shown in Tables 3-8.

Symbols in the tables 3-8:
Method 1 - computer image analysis,
Method 2 - gravimetric method,
δ_x - relative error, %,
SD - standard deviation,
t - t-Student test result,
p - probability value.

Based on the results, the relative errors were used to reject the opportunity to determine the content of a tracer using a combination of two fluorescent substances Tinopal and Uranine. For combinations of these (Series 4 and 6) mean of relative errors ranged from 14.30-41.59% (Tables 6 and 8), which means that they substantially deviated from the assumed verification threshold ≤5%.

Table 3. The results obtained for series 1
Tab. 3. Wyniki uzyskane dla serii 1

Number of sample	Share of tracer, %					
	Maize			Kardi		
	Met. 1	Met. 2	$\delta_{x, \%}$	Met. 1	Met. 2	$\delta_{x, \%}$
1	6.46	6.19	4.42	10.14	9.72	4.33
2	6.5	6.32	2.81	11.26	10.83	3.94
3	6.46	6.84	5.54	13.45	12.84	4.71
4	8.95	9.04	1.00	12.39	11.70	5.87
5	9.15	9.12	0.31	11.61	11.30	2.78
6	7.88	7.82	0.80	11.26	10.67	5.52
7	10.83	11.46	5.49	12.31	11.65	5.67
8	5.96	6.13	2.81	11.28	10.92	3.34
9	8.14	8.47	3.89	13.07	12.71	2.84
10	6.79	7.14	4.87	11.09	11.43	2.99
Mean	7.71	7.85	3.19	11.79	11.38	4.20
SD	1.48	1.61	1.96	0.95	0.89	1.15
t	-0.19		-	0.94		-
p	0.85		-	0.36		-

Source: own work / Źródło: opracowanie własne

Table 4. The results obtained for series 2
Tab. 4. Wyniki uzyskane dla serii 2

Number of sample	Share of tracer, %					
	Maize			Kardi		
	Met. 1	Met. 2	$\delta_{x, \%}$	Met. 1	Met. 2	$\delta_{x, \%}$
1	6.44	6.19	4.10	10.29	10.74	4.19
2	6.27	6.49	3.32	11.81	11.31	4.42
3	6.89	7.22	4.56	10.16	9.67	5.07
4	6.03	6.27	3.80	10.23	9.82	4.18
5	7.96	8.03	0.93	11.49	10.93	5.12
6	7.95	8.22	3.34	9.52	9.83	3.15
7	7.97	8.39	4.98	11.04	11.33	2.56
8	7.49	7.93	5.50	10.72	10.42	2.88
9	7.92	8.09	2.09	11.44	11.87	3.62
10	8.89	9.26	3.97	10.83	10.28	5.35
Mean	7.38	7.61	3.66	10.753	10.62	4.05
SD	0.88	0.97	1.28	0.68	0.71	0.93
t	-0.52		-	0.41		-
p	0.61		-	0.69		-

Source: own work / Źródło: opracowanie własne

Table 5. The results obtained for series 3
Tab. 5. Wyniki uzyskane dla serii 3

Number of sample	Share of tracer, %					
	Maize			Kardi		
	Met. 1	Met. 2	$\delta_{x, \%}$	Met. 1	Met. 2	$\delta_{x, \%}$
1	7.19	7.00	2.69	7.32	7.08	3.34
2	2.83	2.68	5.56	6.79	7.14	4.87
3	9.28	9.04	2.65	10.06	9.53	5.57
4	6.43	6.27	2.58	8.19	8.36	2.04
5	6.16	5.86	5.11	5.92	5.72	3.41
6	9.87	10.13	2.54	9.46	9.45	0.13
7	8.36	8.93	6.40	7.91	7.52	5.21
8	9.16	9.64	4.96	7.61	7.93	3.98
9	11.7	11.89	1.63	8.22	7.87	4.43
10	13.41	13.06	2.66	8.57	8.47	1.19
Mean	8.44	8.45	3.68	8.00	7.91	3.42
SD	2.84	2.91	1.56	1.15	1.08	1.70
t	0.008		-	0.19		-
p	0.99		-	0.85		-

Source: own work / Źródło: opracowanie własne

Table 6. The results obtained for series 4
Tab. 6. Wyniki uzyskane dla serii 4

Number of sample	Share of tracer, %					
	Maize			Kardi		
	Met. 1	Met. 2	$\delta_{x, \%}$	Met. 1	Met. 2	$\delta_{x, \%}$
1	11.15	7.00	59.24	8.58	9.94	13.65
2	8.9	5.51	61.61	8.25	10.40	20.66
3	8.58	8.96	4.23	8.65	12.30	29.68
4	8.78	6.84	28.39	7.83	11.46	31.67
5	9.11	6.54	39.30	9.87	11.24	12.20
6	9.69	8.61	12.61	7.78	10.89	28.55
7	8.71	8.12	7.32	9.87	12.82	23.00
8	9.44	8.80	7.33	7.78	10.64	26.90
9	9.41	10.10	6.83	9.08	12.52	27.47
10	11.11	13.06	14.94	8.21	10.18	19.36
Mean	9.49	8.35	24.18	8.59	11.24	23.31
SD	0.89	2.04	20.89	0.75	0.96	6.38

Source: own work / Źródło: opracowanie własne

Table 7. The results obtained for series 5
Tab. 7. Wyniki uzyskane dla serii 5

Number of sample	Share of tracer, %					
	Maize			Kardi		
	Met. 1	Met. 2	$\delta_{x, \%}$	Met. 1	Met. 2	$\delta_{x, \%}$
1	6.09	6.32	3.68	5.34	5.32	0.43
2	6.97	7.19	3.09	4.63	4.94	6.21
3	6.89	6.54	5.35	4.82	5.02	3.95
4	9.27	9.31	0.45	6.51	6.40	1.66
5	7.58	7.79	2.70	7.59	7.25	4.74
6	8.19	8.50	3.61	8.64	8.93	3.26
7	6.77	6.40	5.72	5.16	5.26	1.95
8	7.46	7.93	5.88	4.95	5.10	2.93
9	7.26	7.93	8.40	4.29	4.15	3.41
10	6.33	6.32	0.12	3.49	3.25	7.33
Mean	7.28	7.42	3.90	5.54	5.56	3.59
SD	0.88	0.98	2.41	1.49	1.53	2.09
t	-0.32		-	-0.03		-
p	0.75		-	0.98		-

Source: own work / Źródło: opracowanie własne

Table 8. The results obtained for series 6
Tab. 8. Wyniki uzyskane dla serii 6

No. of sample	Share of tracer, %					
	Maize			Kardi		
	Met. 1	Met. 2	$\delta_{x,\ \%}$	Met. 1	Met. 2	$\delta_{x,\ \%}$
1	3.79	5.78	34.42	12.02	9.96	20.63
2	3.97	5.24	24.17	13.42	10.10	32.87
3	4.26	5.67	24.87	12.25	12.19	0.47
4	3.98	8.77	54.61	13.1	11.98	9.39
5	4.89	7.52	34.96	12.58	11.68	7.74
6	5.58	8.22	32.16	12.82	10.51	22.01
7	3.02	6.40	52.84	11.85	12.52	5.34
8	3.23	7.38	56.25	11.82	10.53	12.20
9	4.34	7.36	40.99	14.55	12.52	16.23
10	2.02	5.13	60.60	11.47	9.88	16.06
Mean	3.91	6.75	41.59	12.59	11.19	14.30
SD	0.94	1.21	12.81	0.88	1.03	8.91

Source: own work / Źródło: opracowanie własne

For this series no further calculations (statistical comparison tests) of comparable statistics were performed. Significantly better results with the acceptable mean relative error were obtained for the tests with one tracer (maize or kardi) coated with 0.01% solution of Rhodamine B (Tables 1, 2, 3 and 5). The extent of this error ranges in different series from 3.19% to 3.66%. Mean relative error for the second tracer (in this series). covered with the solutions of Tinopal and Uranine ranged from 3.68% to 4.20%. This confirms earlier observations suggesting that the best results in the fluorescence method are achieved by using Rhodamine B [11]. The analysis of the size of relative error for various samples (δ_{xi}) indicated that in some cases the acceptable value was exceeded. However, taking into account the results of statistical comparative analysis we can assume that single small deviations do not affect the final result and confirm the usefulness of the proposed method for the simultaneous determination of the share of two critical components. For the test series 1, 2, 3 and 5 there is no reason to reject the null hypothesis of equality of results obtained in two ways, at the fixed level of significance $\alpha = 0.05$.

4. Conclusions

- The phenomenon of fluorescence excited by ultraviolet radiation can be used for the simultaneous determination of two critical components (maize and kardi) in a compound grain mixture.
- Key components must be stained with fluorescent substances different in terms of the emitted colour. One of them is the Rhodamine B, while the other may be Uranine or Tinopal.
- The results for series 1, 2, 3 and 5 showed no statistically significant difference between the results using fluorescence (method 1) and verification method (method 2).

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