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Research Article

Application of the IR spectrometry method in the screening study of various oat species

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BACKGROUND: The infrared reflection spectroscopy application method for rapid assessment of biochemical parameters in various types of oats is shown. On the basis of the biochemical data obtained in the laboratory of VIR, calibration models of protein, oil and starch content were constructed.

AIM: The aim of the study is to develop an express method of near-infrared spectroscopy (NIRS) spectroscopy to determine the main biochemical parameters in oat seeds and to build calibration models for the MATRIX-I IR analyser to quantify the mass fraction of protein, oil and starch in oat seeds based on data obtained by traditional methods.

MATERIALS AND METHODS: Biochemical quality indicators (protein, oil, starch) were studied on seeds of filmy oats (*Avena sativa* L.) grown in 2015–2016 in the North-Western Region of the Russian Federation. Calibration models for the determination of protein, oil and starch in oat seeds (98 samples, harvest 2014–2015) were developed for the MATRIX-I IR analyzer by Bruker Optics (Germany). Values obtained by traditional chemical methods of analysis were used to construct calibration models. Oat seed oil was determined by the Soxlet method, protein by the Kjeldahl method, starch by the Evers polarimetric method. All indicators were recalculated for dry weight.

RESULTS AND CONCLUSION: The reliability of the developed models was checked by the results of protein, oil and starch determination in the seeds of the test batch according to the indicator of the calibration correctness. The data obtained using the calibration curve on the MATRIX-I device had no significant differences with the results of chemical studies. Therefore, calibration can be used for screening analysis for protein, oil and starch content in oat samples. This method allows you to save valuable material, increase labour productivity due to the speed of obtaining data, does not require reagents and is safe.

Keywords: oats; calibration model; IR spectroscopy method; protein; oil; starch.

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Научная статья

Применение метода инфракрасной спектрометрии в скрининговом исследовании различных видов овса

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Актуальность. Проведена оценка биохимических показателей у различных видов овса методом спектроскопии ближнего инфракрасного отражения. На основании полученных в лаборатории ВИР биохимических данных были построены градуировочные модели содержания белка, масла и крахмала.

Материалы и методы. Биохимические показатели качества (белок, масло, крахмал) изучали на семенах пленчатого овса (*Avena sativa* L.), выращенного в 2015–2016 гг. на Северо-Западе Российской Федерации. Градуировочные модели по определению белка, масла и крахмала в семенах овса (98 образцов, урожай 2014–2015 гг.) разработаны для ИК-анализатора MATRIX-I (Bruker Optics, Германия). Для построения градуировочных моделей использовали значения, полученные традиционными, химическими методами анализа. Биохимический состав масла у семян овса определяли методом Сокслета, белка — методом Кьельдаля, крахмала — поляриметрическим методом Эверса. Все показатели пересчитывали на сухой вес образца.

Результаты и заключение. Достоверность разработанных моделей проверяли по результатам определения содержания белка, масла и крахмала у семян проверочной партии по показателю правильности градуировки. Данные, полученные с помощью калибровочной кривой на приборе MATRIX-I, не имели достоверных различий с результатами химических исследований. Следовательно, калибровка может быть использована для скрининг-анализа на содержание белка, масла и крахмала в образцах овса. Данный метод позволяет сохранить ценный материал, повысить производительность труда за счет оперативности получения данных, не требует реактивов и безопасен.

Ключевые слова: овес; градуировочная модель; метод ИК-спектроскопии; белок; масло; крахмал.

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ЭКО•ВЕКТОР

BACKGROUND

Sowing oats (*Avena* L.) is a moisture–loving plant unpretentious to soils and climate with a relatively short growing season. It has lower requirements for summer heat and higher resistance to rain than other cereals, such as wheat, rye or barley, which is especially important for areas with cool and humid summers, including North-Western Europe [1].

Two subspecies are distinguished in the varietal diversity of the oat species — filmy oats (*A. sativa* subsp. *sativa* L.) and naked oats (*A. sativa* subsp. *nudisativa*) [2].

In 2016, the yield of oats (the number of quintals per 1 hectare) in Russia amounted to 17.3 c/ha, which is 8.1% or 1.3 c/ha higher than in 2015. In relation to 1990, the yield of oats increased by 16.9% or 2.5 c/ha. At the same time, the average harvest in Russia from 1990 to 2018 was 15.4 c/ha. The highest yield of oats in 2016 was observed in the Krasnodar Territory — 34.1 c/ha. The Leningrad region is located in the 7th position (24.8 c/ha). Compared to 2015, a decrease of 10.5% or 2.9 c/ha was identified [3].

Oats is one of the most popular forage crops in world grain production, is cultivated as an annual crop, and has a number of valuable properties that meet the requirements for "functional nutrition" products, as well as allowing it to be used for fodder and medical and preventive purposes [4, 5].

The value of this crop used in the food and feed industries is determined by the quality of grain and its chemical composition. Oat grain has a high nutritional value, contains unsaturated fatty acids, basic mineral elements, globular proteins, starch and non-starch polysaccharides, β-glucans, in particular, are characterised by the presence of a variety of chemicals that exhibit antioxidant properties [6].

The chemical composition of oat grain, which determines its biological value, is highly variable depending on the genotype and growing conditions. The range of interport variability in protein content is 8.3–20.7% [7]. Thus, oats can be grown as a protein crop. In addition, studies conducted at Vavilov All-Russian Institute of Plant Genetic Resources (VIR) have shown the important role of oats as a wheat substitute for the organisation of a gluten-free nutrition diet for people suffering from wheat protein intolerance or celiac disease [8]. The starch content in the grain ranges from 23.7% to 69.5%, β-glucans — from 2.0% to 7.5% [9–11]. Compared with other grain crops, oat grain contains a relatively large amount of valuable oil in its composition, rich in so called essential fatty acids for humans: unsaturated — linoleic (C18:2, ω-3) and linolenic (C18:3, ω-3), as well as arachidonic (C20:4, ω-6), together making up the so-called vitamin F. The oil content in the grain can range from 2.0% to 10.6% and higher [1, 12]. Varieties with a relatively high oil content (more than 6%) are: Abel, Siberian Nudibranch, Percheron and Vladyka: 6.6, 8.1, 15.7 и 17.3%, respectively [13].

The study of the variability of various indicators of oats quality gives a versatile characteristic of the species and varietal diversity of the world's VIR oats collection, allows us to identify samples with the most pronounced manifestation of these signs for more effective use in the food industry and practical breeding and become the basis for forecasting in the work of breeders.

According to the methodological recommendations [14], the term "sources" can be applied to the best samples (but not studied on this basis by genetic methods). The world collection of oats in VIR institute has about 14,000 samples and their potential for use in various fields has not yet been sufficiently studied. Therefore, there is a need for screening analyses of large sample batches. This task is best solved with the use of infrared spectroscopy, which makes it possible to conduct biochemical studies of a large amount of seed material quickly and practically without loss. Therefore, it is permissible to make measurements, both in flour and in a medium-sized whole grain, having previously built an appropriate grain calibration model. This method allows rapid evaluation of breeding material with a productivity of more than 100 samples per day [15, 16].

The aim of the study is to develop an express method of near-infrared spectroscopy (NIRS) spectroscopy to determine the main biochemical parameters in oat seeds and to build calibration models for the MATRIX–I IR analyser to quantify the mass fraction of protein, oil and starch in oat seeds based on data obtained by traditional methods.

MATERIALS AND METHODS

Samples of oats from the VIR collection served as the material for constructing calibration models. The formed set of oats was grown in the conditions of the North-West of the Russian Federation (Leningrad Region). Sowing, observations and harvesting were carried out in accordance with the methodological recommendations for the study and preservation of the global collection of barley and oats [17] in the conditions of the scientific and technical base of the Pushkin VIR experimental field station (59°71' c. w., 30°38' v. d.) in 2014–2016 and were collected at the maturity One each sample was sowed on 1 m^2 in a twofold repetition, the sowing time was optimal for the research region. the plot area was 1 m^2.

The soil of the experimental field was sod-slightly podzolic, sandy loam in texture, neutral acidity amounted — 7.1–7.6 pH. The thickness of the humus horizon ranged from 23 to 47 cm, and the humus content was 2.1–3.0%. The quantity of mobile forms of potassium was average, and phosphorus — high. The climate in this agro-climatic region is characterised as moderately warm with cool summers in some years. The warmest month of the year is July with an average long-term air temperature of 16.5–17.7 °C. The sum of positive temperatures is 2100–2300 °C. The period with a temperature above 10 °C lasts 105–115 days. The amount of precipitation during the growing season is 550–600 mm per year.

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Weather conditions during the years of study were obviously different: average temperatures, precipitation and hydrothermic coefficient (GTC) for July and August in 2014, 2015 and 2016 are presented in Table 1.

According to the sum of temperatures, the warmest year for the studied period was 2014, and the coldest was 2016. By the amount of precipitation, on the contrary, 2016 turned out to be the wettest year, and 2014 was the driest, which is also characterised by the GTC.

For the construction of calibration models, film samples of oats grown in 2014–2015 years were used (Appendix, Table 1). Among these samples the species: *A. sativa* L. (23 samples), *A. abyssinica* Hochst. (3 samples), *A. strigosa* Schreb. (3 samples) and 20 samples of interspecific hybrids of *A. sativa* × *A. byzantina* were used (98 samples in total — 49 in 2014 and 49 in 2015). All the presented types of oats are film samples and differ significantly in biochemical characteristics, which is important for calibration models construction.

To verify the correctness of the calibration models other samples of 2015–2016 oat grain of the following species and interspecific hybrids were selected: *var. aurea* (6), *var. mutica* (6) and *A. sativa* × *A. byzantina* (16) (a total of 28 samples) (Table 2).

Table 1. Weather conditions during the maturation of oat grain for 2014–2016

Month	Decade	2014			2015			2016		
		Sum t, °C	Precipitation amount, mm	GTC*	Sum t, °C	Precipitation amount, mm	GTC *	Sum t, °C	Precipitation amount, mm	GTC*
July	I	195	7.4	0.38	193	52.6	2.73	180	77.5	4.3
	II	232	10.9	0.47	171	37.3	2.18	192	42.1	2.19
	III	267.3	3.1	0.12	204.6	26.3	1.29	236	54.6	2.31
Per month		694.3	21.4	0.31	569	116.2	2.04	608	174	2.86
August	I	247	1.0	0.04	206	8.8	0.42	197	31.0	1.57
	II	193	23.7	1.23	191	13.8	0.72	170	106.7	1.23
	III	165	44.3	2.95	221	12.7	0.57	198	36.6	2.95
Per month		605	69	1.14	618	35.3	0.57	565	174.3	3.08
Total for 2 months		1 299.3	90.4	1.45	1187	151.5	2.61	1173	348.3	5.94

*The hydrothermal coefficients (GTC) significantly differed in different years, calculated by the formula: (sum of precipitation (mm) × 10) / sum of temperatures). The average annual GTC in 2014 was 0.92; in 2015 — 1.04; in 2016 — 1.8.

Table 2. Samples of oats from the 2015-16 harvest, selected for calibration models testing

Sample number *	VIR catalog number VIR	Origin	Variety	Species
1, 2	15547	Leningrad Region	Precocious 1	*A. sat.* × *A. byz.*
3, 4	15548	Leningrad Region	Precocious 2	*A. sat.* × *A. byz.*
5, 6	15549	Leningrad Region	Medium-ripened 1	*A. sat.* × *A. byz.*
7, 8	15550	Leningrad Region	Medium-ripened 2	*A. sat.* × *A. byz.*
9, 10	15551	Leningrad Region	Late maturing	*A. sat.* × *A. byz.*
11, 12	15523	China	Bai Yan 6	*A. sat.* × *A. byz.*
13, 14	15524	China	Bai Yan 7	*A. sat.* × *A. byz.*
15, 16	15529	Brazil	Ufrgs 1	*A. sat.* × *A. byz.*
17, 18	15565	Ulyanovsk Region	Kenter	*var. mutica*
19, 20	15498	Sverdlovsk Region	Uralets	*var. mutica*
21, 22	15499	Sakha Yakutia	Vilemskiy	*var. mutica*
23, 24	15500	Belarus	Mirt	*var. aurea*
25, 26	15521	China	Z 0585	*var. aurea*
27, 28	15487	Brazil	Urs Guria	*var. aurea*

* odd number — 2015; even number — 2016.

Sample preparation and analysis methods. For the analysis, the grain was grinded using a CM 290 Cemotec disc mill (FOSS, Sweden). Samples of 40–50 g were formed from the obtained samples in metal boxes, which were kept at room temperature from 3 to 5 days to equalise them in humidity and temperature.

The biochemical analysis was carried out in the Department of Biochemistry and Molecular Biology according to the VIR methodology [18].

The protein content was determined by the Kjeldahl method using a semi-automatic analyzer Kjeltec 2200 (FOSS, Sweden) with an automatic distillation unit. The total protein content was calculated by nitrogen content with a coefficient of 5.7. The indicators were expressed in "% on dry matter". The oil content was determined by the mass of the dry fat-free residue using the Soxlet apparatus and petroleum ether as a solvent (boiling point 40–70 °C). Starch content is determined by the Evers polarimetric method using an automatic polarimeter SAC-i (ATAGO, Japan). The recalculation coefficient for oats is 181.3. Humidity was determined by drying the samples at 100–102 °C to a constant mass.

According to the manual for the MATRIX-I IR Fourier spectrometer from Bruker Optics (Germany), spectra for ground oat samples (flour module) were obtained using OPUS software.

The obtained spectra were recorded in the range of 3600–12800 cm^{-1} with a resolution of 2.0 cm^{-1}. The spectra of each sample were recorded in three repetitions with interspersing in a cuvette with a diameter of 51 mm (weight 20 ± 1 g).

Calibration models were calculated by the method of multivariate analysis on the spectra of calibration samples with known values of the parameters to be determined, in accordance with the operating instructions of the IR analyzer and software.

Calibration samples – samples of flour obtained from oat seeds with known values of mass fractions: protein, oil, starch, moisture, established in accordance with the methods adopted in the seed biochemical analysis. The values of the mass fractions of protein, oil, and starch for calibration purposes were calculated at the actual humidity of the calibration samples.

Ninety-eight calibration samples were used to calibrate the IR analyzer for each indicator. The samples were selected in such a way that the values of the mass fractions of the analysed indicator in the calibration kit were evenly distributed over the entire measurement range. The value of the correlation coefficient of the calibration model was at least $r = 0.8$.

Analysis. The well-mixed analysed sample was loaded into the cuvette with a spatula in accordance with the instructions for the device and slightly compacted taking in the account that it must be placed in the measuring cuvette in the same way as it was done when registering the spectra of calibration samples since the intensity of the IR spectra is greatly influenced by the density of the packing material in the cuvette. It is not recommended to pour the stopper from the vessel, since the sample is divided into fractions and the measurement accuracy decreases. Also, when grinding new grain samples, it is important to use the same mill that was used to obtain calibration samples.

The mass fraction of the measured indicator (except moisture) in terms of absolutely dry matter X_1,% was calculated by formula 1:

$$X_1 = \frac{X \cdot 100}{100 - W}, \quad (1)$$

where X is the mass fraction of the measured indicator corresponding to the reading of the device, %; W — the mass fraction of moisture and volatile substances, %.

For the final measurement result was taken as the arithmetic mean of three parallel definitions performed under repeatability conditions and satisfying the acceptance condition. The result was rounded to the first decimal place.

Statistical data processing was performed using the Microsoft Office 2016 software package.

The BIC spectroscopy method is based on recording the reflection spectra of the analysed samples in the near infrared region (from 780–2770 nm) and determining the mass fractions of protein, oil, starch, moisture and other indicators in them, depending on the developed calibration models, according to which the values of the indicators are calculated in the future.

Determining error of this method in absolute units is not more than 0.5% and slightly exceeds the error of arbitration methods, but is incomparable in labour productivity.

RESULTS AND DISCUSSION

The spectral data are greatly influenced by the conditions, zone and year of cultivation, as well as the genotypes of the samples. Therefore, the formation of calibration and verification batches of samples for calibration models for determining the biochemical parameters of seeds must be carried out every year, periodically adding new samples, especially those that go beyond the defined boundaries, and recalculating the calibration model again.

The IR spectra for oat grain obtained by scanning the samples are shown in Fig. 1.

During two years of research, 98 samples with a protein mass fraction from 10.7 to 20.4%, oil — 3.1–7.4% and starch — 27.3–54.5% were selected for the construction of calibration models. The moisture content in the samples is a relatively stable indicator with a small range of variability from 91.91% to 92.75% and averages 92.3%.

Studies have shown that combining all samples into one calibration model makes it possible to increase the stability of such a model due to the widest possible range of protein variability, oil and starch content in the grain.

At the stage of calibration models development the OPUS software for the MATRIX–I IR analyzer was used to

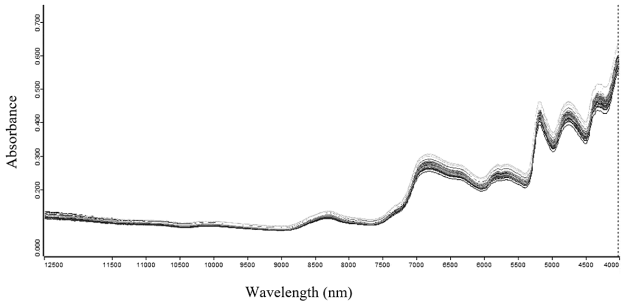

Fig. 1. IR spectra of the studied oat grain samples

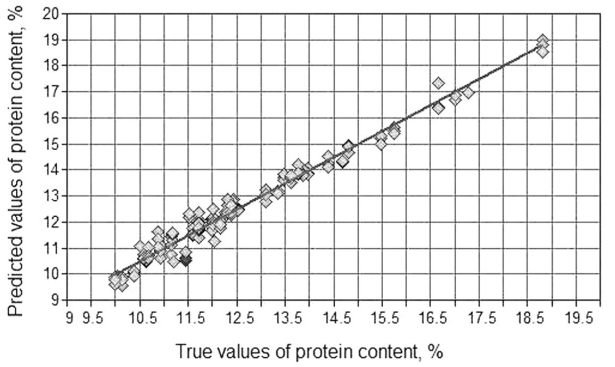

Fig. 2. Graph of predicted values of protein content in oats compared with the true values of protein conten of the Oat_Protein calibration model

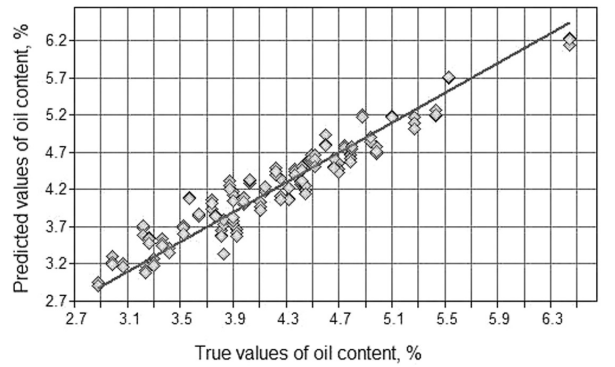

Fig. 3. Graph of predicted values of oil content in oats compared with the true values of oil content of the Oat_O il calibration model

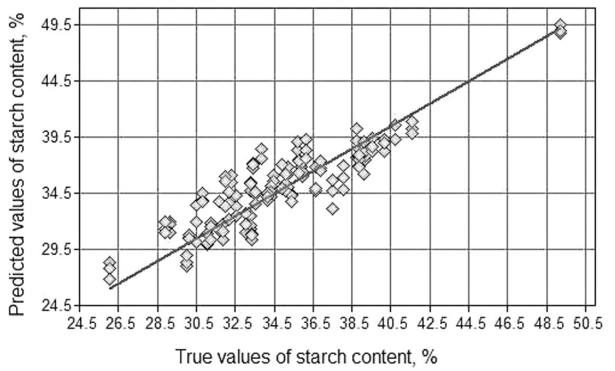

Fig. 4. Graph of predicted starch content values in oats compared to the true starch content values of the Oat_Starch calibration model

divide the calibration batch of samples into two equivalent parts — calibration and test (49 samples each). During the model development, 294 spectra from calibration samples were calculated using different algorithms and tested on test samples. The obtained models were evaluated by four main factors: the magnitude of the RMSEP calibration error, the number of ranks, the spectral range and the pre-processing method.

As a result of the analysis of spectral data by the method of pre-processing of the first derivative + vector normalization (protein and starch) and elimination of constant displacement (oil) in three ranges: 11069.3–3757.6, 11053.9–3773.1 and 11131–3788.5 cm^{-1}, calibration models "Oat_Protein", "Oat_Oil", "Oat_Starch" were constructed.

Spectra's with a standard deviation higher than the permissible one are shown on the graph with red dots. Such samples are excluded. So, it should be noted that in the process of models developing part of the registered spectra was removed from the spectra table in order to increase the coefficient of determination (R^2) — 18 samples for protein, 3 for oil and 2 for starch (out of the total 294).

For the protein model (Fig. 2), with a high coefficient of determination R^2 — 97.37, the number of ranks in the multivariate analysis is 10, the mean square error of prediction (RMSEP) — 0.323%, which gives confidence in the reliable repeatability of the obtained results of oat flour.

The RPD indicator (Residual Prediction Deviation — the value of the residual prediction deviation for the rank) of the developed calibration model evaluates the stability of the obtained dependence. According to the assessment adopted for the OPUS program, the magnitude of this value in the range from 6.0 to 7.9 qualifies as a "very good dependence" [19–22]. The developed Oat_Protein calibration model for the determination of protein in oat flour has an RPD value of 6.21.

For the oil model (Fig. 3) R^2 is 92.03, the number of ranks in the multivariate analysis is 8, RMSEP is 0.206%, which corresponds to good repeatability of the results obtained. The RPD of the developed Oat_Oil calibration model for the determination of oil in oat flour has a value of 3.58, which is below the optimal level. To increase the accuracy of this calibration model, its expansion and additional sample's introduction followed by recalculation and re-construction of the model are required. The peculiarity of these models is that they can be constantly supplemented and improved by introducing the values of new samples into them.

For the starch model (Fig. 4) R^2 is 79.9, the number of ranks in the multivariate analysis is 11, RMSEP is 1.8%. The RPD of the developed Oat_Starch calibration model for determining starch in oat flour has a value of 2.25, which is below the optimal level.

98 samples were used to construct the calibration model. To verify the correctness of the model, new samples of oats (28) which were not included in the original batch of samples (98) were used.

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The discrepancy between the IR analyzer readings and the values determined by standard methods was calculated. The average deviation $\Delta\bar{X}$ value was calculated according to formula 2 [23]:

$$\Delta\bar{X} = \frac{\Sigma\left|X_{IR} - X_{St}\right|}{n}, \quad (2)$$

where X_{IR} is the percent value of the indicator obtained by IR spectroscopy; X_{St} — the value obtained by the standard method; n — number of samples used to check the calibration (28).

The value $\Delta\bar{X}$ does not exceed the error of the standard method (for protein — 0.11, for oil — 0.13), but slightly exceeds for starch — 0.76% (Appendix, Table 2).

The relative difference between the chemical methods of grain analysis and the physical method based on IR spectroscopy in the middle does not exceed 2–4% (Appendix, Table 2).

CONCLUSION

Near-infrared spectroscopy was used to evaluate the biochemical components in flour obtained from whole grain oats. It allows to significantly reduce the time for determining biochemical parameters, since it does not require preliminary sample preparation, except for grain grinding, and the analysis itself takes several minutes. After grinding, the seed samples become homogeneous both in structure and colour; therefore, the determination error indicators decrease. However, for breeding work, it is necessary to preserve valuable material and, on the other hand, to increase labour productivity by reducing the operation of grinding seeds.

On the example of oat samples, calibration models for the determination of protein, oil and starch were obtained. The obtained models were tested on 28 samples that were not used in the construction of the calibration dependence, with known values of the analysed indicators established by standard chemical methods. The value did not exceed the errors of the standard method (for protein — 0.11, for oil — 0.13) and slightly exceeds for starch — 0.76.

BIC spectroscopy is an express method that allows to perform mass screening analyses with the possibility to obtain results for several indicators at once, with a given repeatability and standard deviation after scanning the sample. Using the necessary calibration models, it is possible to determine both chemical and physical parameters of the samples, there is no need for chemical reagents, and the analysis itself takes a few minutes. In addition, ready-made calibration models can be easily transferred from one device to another.

ADDITIONAL INFORMATION

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Conflict of interest. The authors declare no conflict of interest.

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