Adaptation of the rapid near-infrared (NIR) spectroscopy technique to determine the mass fraction of protein and moisture in gluten-free bakery products

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Abstract. Analysis of protein in food products is typically performed using a time-consuming and labor-intensive Kjeldahl method. The NIR method is allowed to determine the mass fraction of not only protein, but also moisture, fat, and sugar in one sample within 2 min. The NIR method is indirect; therefore, appropriate calibration models should be developed for each group of analyzed products. We performed a series of laboratory baking tests for gluten-free bakery products; the protein content in the products varied and depended on the ingredients included into the formula: rice, buckwheat and corn flour, starch and soy isolate. Continuous spectra were recorded using a NIR analyzer that operates in the wavelength range from 1400 to 2500 nm. It was shown that after validation for a specific device, the developed calibration models yield the root-mean-square deviation of 0.29 when determining the protein mass fraction and that of 0.46 when determining the moisture mass fraction.

1 Introduction

Bread is one of the most popular foods in the human diet. Information on its nutritional value on the label must be reliable. Currently, bakery products are largely produced from flour containing gluten (gluten protein). People with celiac disease should exclude gluten-containing foods. Gluten-free bakery products are typically made from flour of the following grains: rice, corn, buckwheat, quinoa. Supplementary protein sources can include soy, pea, eggs, milk, etc. [1-3].

The mass fraction of protein is an essential indicator of the quality of gluten-free products; it is provided both per 100 g of the whole product with a crust, and with respect to dry matter (DM). The protein content is determined by chemical methods (mainly by the Kjeldahl method) through a lengthy, multi-stage process. The moisture content can be easily determined by thermogravimetric method, yet it is time consuming and requires additional

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equipment. It is shown that the NIR method is the best option for analysis. It implies recording the reflectance spectra of analyzed samples in the near-infrared region from 750 to 2500 nm (from 13333 to 4000 cm^{-1}) and automatic calculation of the mass fraction of a particular component using pre-developed calibration models, elaborated for a representative sample of bakery products with the content of these components estimated by standard chemical and physico-chemical methods. The NIR method employs one device to quickly determine the content of protein, moisture, fat, and sugar [4-8].

In the NIR region, the signals are of low intensity (two to three orders of magnitude less than that of the signals in the IR region), and a large number of overlapping signals complicate determination of the baseline. Yet, in the food industry, NIR analyzers are more common than IR analyzers, since full-fledged IR spectrometers are several times more expensive than NIR analyzers, they are much more difficult to operate and require highly skilled operators and expensive consumables [4, 5, 9, 10].

NIR analyzers are manufactured in Russia and can be used to determine the quality indicators of a wide range of food products and raw materials used for their production. Calibration models have already been developed for the analysis of grain and flour, milk and products of its processing, oilseeds and raw materials for animal feed production. Until recently, the lack of a regulatory framework and appropriate calibration models in Russia hampered the use of the NIR method for the analysis of bakery products, including gluten-free products. Adaptation of the NIR method and its inclusion in the relevant GOSTs will allow its use in plant bakeries, and simplify and speed up the analysis of raw materials (flour) and finished products.

2 Research objects and methods

The study objects were gluten-free products based on rice, buckwheat and corn flour, baked at the Scientific Research Institute for the Baking Industry in accordance with the model formulas.

The mass fraction of moisture was determined by drying samples of crushed products with a crust to a constant mass at a temperature of 130 °C. The process was performed using drying cabinets SESH-3M and ASESH 8-2, and a AND MX-50 moisture analyzer.

Continuous spectra were recorded using an INFRASCAN-3150 IR analyzer operating in the wavelength range from 1400 to 2500 nm (from 7140 to 4000 cm^{-1}). According to the literature data, NIR analyzers are calibrated for protein analysis using the results obtained by the Kjeldahl method as a reference [4, 11]. The Kjeldahl method is based on the disruption of peptide bonds in proteins, followed by the release of nitrogen molecules, and their quantitative analysis using titration. Nitrogen to protein conversion is performed using the K coefficient typically equal to 6.25, which corresponds to 0.16 g of nitrogen per gram of protein. In different literature sources, the K coefficient values for one particular raw material may differ. For result processing, we took K equal to 6.25 for buckwheat, corn and soybeans, and 6.0 for rice. For finished gluten-free products (with regard to the type and amount of raw materials in the formulas), the K value varied from 6.1 to 6.2.

The NIR method is an indirect method, and its validity strongly depends on the reference method used as a basis to develop a calibration model. Therefore, the validation process for a particular instrument should be maximally complete.

To develop calibration models, 20 versions of gluten-free products were baked with an estimated mass fraction of protein varying from 1.7% to 8.7% (Table 1).

Table 1. Estimated and actual protein content in gluten-free bread baked in accordance with model formulas.

No.	Dosage, %		Flour	Nitrogen content, % in terms of DM	Protein content, % in terms of DM	Protein content, g per 100 g of the product	
	soy protein	starch				estimated	Kjeldahl
1	0	65	rice + buckwheat	0.5880	3.59	1.75	**1.93**
2	0	55	rice + buckwheat	0.6834	4.17	2.33	**2.12**
3	1	57	rice + buckwheat	0.8747	5.34	2.41	**2.69**
4	2	56	rice + buckwheat	1.0188	6.21	2.83	**3.12**
5	4	58	rice	1.0001	6.10	3.16	**2.93**
6	5	60	rice	1.0945	6.68	3.39	**3.13**
7	6	58	corn	1.2806	7.81	3.97	**3.75**
8	8	57	rice	1.5046	9.18	4.67	**4.46**
9	8	57	rice	1.5278	9.32	4.58	**4.35**
10	9	56	rice	1.6732	10.21	4.99	**4.80**
11	10	60	rice	1.7219	10.68	5.24	**5.05**
12	11	59	corn	1.9004	11.78	5.78	**5.68**
13	11	44	rice +corn	2.0551	12.74	6.26	**6.26**
14	12	43	rice	2.2026	13.66	6.47	**6.40**
15	12	38	rice + buckwheat	2.4545	15.22	6.77	**7.14**
16	13	37	rice + buckwheat	2.5944	16.09	7.15	**7.50**
17	14	36	corn	2.5291	15.68	7.68	**7.52**
18	15	30	rice	2.7402	16.99	7.97	**7.98**
19	15	24	rice + buckwheat	3.0428	18.87	8.34	**8.89***
20	16	23	corn	2.9454	18.26	8.70	**8.44***

3 Results and discussion

In the laboratory of EKAN LLC, NIR spectra of the gluten-free products were recorded and compared with those obtained by the Kjeldahl method. The same samples (Table 1) were used to develop the moisture calibration model. For expansion of the spectral range, the samples were either dried or supplemented with a certain amount of water. The device operation speed was reduced 4 fold to obtain more consistent results when measuring the base. The total size of the base for calculations attained approximately 600 measurements. Continuous spectra were recorded, since the device operating at several fixed wavelengths provided analysis results of low accuracy.

During measurement, the NIR analyzer heats up. During routine measurements (2 min), samples do not dry out much, but for calibration model development, sample drying poses significant problems. This can be clearly seen when determining the mass fraction of moisture by the NIR method (vertical rows of points in the graph).

Prior to application, the calibration model must be validated on an individual sample, which is considered as a representative sample for numerous analyzed samples. Figures 1 and 2 show the measurement results for the mass fraction of protein and moisture obtained by the developed calibration models. A strong correlation can be observed between the mass fraction of protein obtained by the Kjeldahl method and that obtained by the NIR method. For moisture, the correlation is much lower, but it remains acceptable.

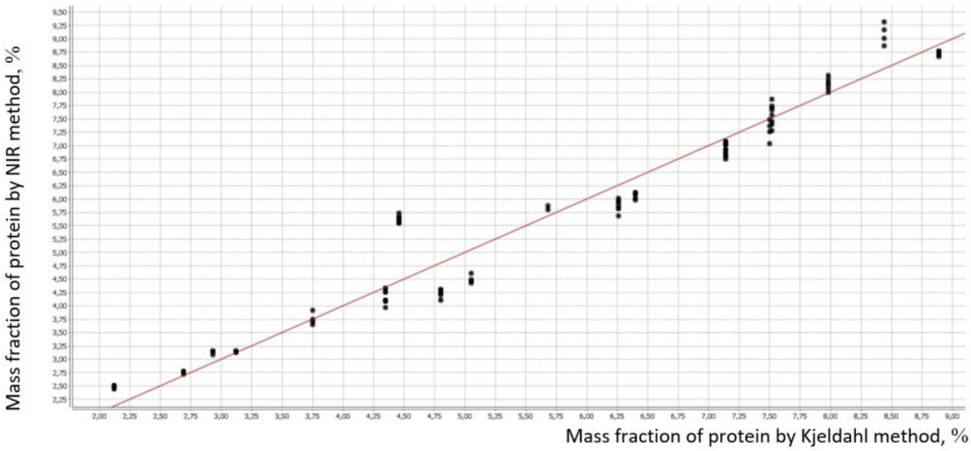

Fig. 1. Correlation between the analysis results on the mass fraction of protein in gluten-free bread obtained by the Kjeldahl method and those obtained by the NIR method.

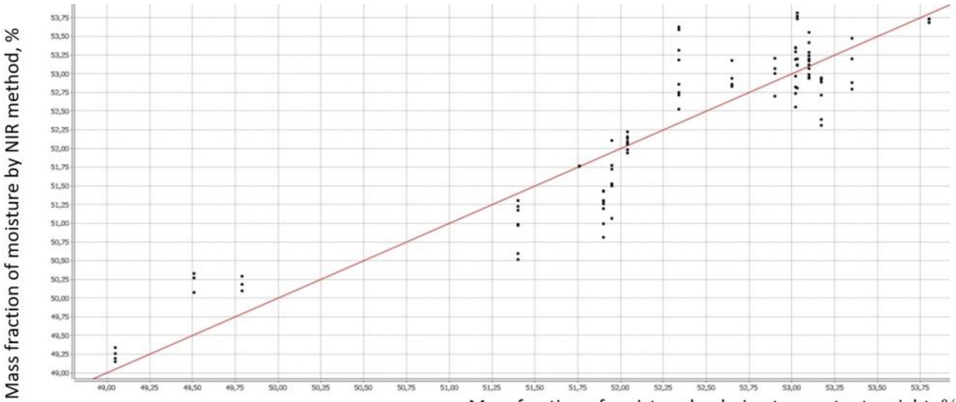

Fig. 2. Correlation between the values of the mass fraction of moisture calculated by drying to constant weight and those obtained by the NIR method.

Table 2 summarizes the NIR method errors in the co-determination of protein and moisture content in gluten-free products.

Table 2. The NIR method error in determining the mass fraction of protein and moisture in gluten-free products calculated by different methods (primarily by multiple regression).

	Mathematical processing of the results					
	For protein mass fraction			For moisture mass fraction		
	Regressio n	PCR	PLS	Regressi on	PCR	PLS
Correlation coefficient	0.99	0.97	0.99	0.92	0.85	0.88
Root-mean -square deviation	0.29	0.46	0.29	0.46	0.61	0.55
Sum of module coefficients	4584	2450	4682	8154	3787	2376
Repeatability (all samples)	0.14	0.10	0.15	0.24	0.30	0.28
Sensitivity (all samples)	0.98	0.97	0.97	0.89	0.91	1.03

4 Conclusion

The study yielded NIR calibration models for the determination of protein and moisture content in gluten-free bakery products (wavelength of 1400 to 2500 nm). It was shown that the NIR method provides an acceptable accuracy of the analysis results. For protein, the correlation coefficient (CC) is 0.99, and the root-mean-square deviation (RMSD) is 0.29, which indicates a good performance of the developed calibration model. For moisture, CC is 0.92, and RMSD is 0.46. The developed calibration models expand the scope of the NIR method, and simplify and speed up the analysis of the chemical composition of gluten-free bakery products.

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