DETERMINATION OF OIL CONTENT IN WHOLE MILK THISTLE SEEDS USING FOURIER TRANSFORM NEAR – INFRARED (FT – NIR) SPECTROSCOPY

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ABSTRACT

Our objective was to create a calibration model for rapid determination of the oil in whole Milk Thistle seeds using the method Fourier Transform Near - Infrared (FT-NIR) Spectroscopy. The seeds from different distributors and dealers from several European countries and from the harvests 2010, 2011 and 2013 were processed by Soxhlet extraction in Det-Gras N instrument and its FT-NIR spectra were collected. The calibration model was created using Partial Least Square (PLS) chemometric analysis – and was successfully tested with the validation samples (spectra not included in the calibration model).

Key words: milk thistle, rapid determination, seed oil, Fourier Transform Near - Infrared (FT-NIR) Spectroscopy, soxhlet extraction

INTRODUCTION

Milk thistle (*Silybum marianum*) is a medicinal plant from the family *Asteraceae*. It is not demanding as for cultivation conditions and its cultivation area is still increasing.

Milk thistle seeds are used for production of the silymarin complex, which is popular for its therapeutic effect on regeneration of liver cells. The silymarin complex is composed of flavonolignans. The oil generated during the production of the silymarin complex is a secondary product [1]. The seeds contain about 22 % or even more oil, which is similar to many vegetable oil seeds. The oil contains large amounts of unsaturated fatty acids (linoleic and oleic), phospholipids, sterols, triglycerides and alfa-tocopherol (vitamin E) [2,3]. The oil is used in animal nutrition, cosmetology and dermatology for its specific properties [1].

For the determination of the oil content in seeds it used to be necessary to use conventional extraction techniques, which cause large consumption of chemicals and destruction of the sample. Therefore, the calibration model using FT-NIR was developed.

The FT-NIR method is based on measuring the transmittance or reflectance of radiation at the wavelengths from 800 nm – 2500 nm (12500 - 4000 cm^{-1}) of different chemical groups present in the sample. It has been considered favourable for many applications in the food, agricultural, pharmaceutical, medical, and plastic industries for quality control and assurance measurements [4,5].

The calibration model was developed and verified for the determination of oil in Milk thistle seeds by the FT-NIR method using chemometric analysis – Partial Least Square (PLS), which was composed of spectra of Milk thistle seed samples from different distributors and dealers from several European countries from the harvests 2010, 2011 and 2013 and tested successfully with the spectra of Milk thistle seeds not included in the calibration model.

This calibration model was able to identify the amount of oil in whole seeds in minutes, without the use of any harmful extraction solvents and sample destruction.

MATERIAL AND METHODS

SAMPLES: Samples were obtained from different European dealers and distributors and from the harvests 2010, 2011 and 2013.

CHEMICALS: Petroleum ether from Verkon was used as the extraction solvent.

EXTRACTION METHOD: For oil extraction from seeds Soxhlet extraction was used. The extraction was carried out on Det-Gras N instrument [6]. The extraction using this machine is faster than the classical Soxhlet apparatus.

3 g of the sample were weighed, ground in mortar and given to the extraction thimble and 50 ml of the extraction solvent was added to the instrument. The solvent was heated to reflux (120°C).

The sample was extracted in the extraction solvent for 15 min as the first extraction step (called boiling step). During the second step (called rinsing step) the sample was extracted in solvent vapours for 45 minutes and then the third part of the extraction (called Solvent recovery phase) followed, when the extraction solvent was renewed, this took 10 minutes. The rest of extraction solvent was evaporated in the drying oven at 85°C. The clean oil was weighed.

The extraction of the sample was repeated three times. After the first and second extraction the sample was removed from the thimble and was processed again. For the second and third

extraction, the second part of extraction took only 30 minutes. From every sample, two sample weights were taken for the triplicate extraction.

FT-NIR METHOD: All FT-NIR spectral data were collected in transmission mode using the cell that allowed direct analysis of whole grains in FT-NIR spectrometer (Magna-IR spectrometer 550 - detector InGaAs, beamsplitter CaF_2) by Nicolet. Every sample was measured in triplicate and the average value was computed from the spectral data. The spectral data were collected over the range 11 446 – 4 000 cm^{-1} with baseline type – linear removed (resolution 8 cm^{-1}), background: 64 scans, sample: 64 scans) at room temperature. The optimal range was very important, it was selected as 11 446 – 4 000 cm^{-1}, which is almost whole spectrum, because the spectrum has not shown any high divergence. Instrument control and initial data processing were performed using OMNIC 8 software. Collected spectra were evaluated by chemometric analysis – Partial Least Square (PLS).

The method used for constructing predictive models was the one suitable for cases when the explanatory variables are many and highly collinear. It may be used with any number of explanatory variables, even for more than the number of observation [7].

The resulting calibration model was evaluated in terms of loading vectors, Root-Mean-Square error of Calibration (RMSEC), Root-Mean-Square error of prediction (RMSEP), Root-Mean-Square Error of Cross-Validation (RMSECV), correlation coefficient of determination (R) and value for the calibration model. RMSECV is an estimate of the magnitude of error expected when independent samples are predicted using the model.

RESULTS AND DISCUSSION

The calibration model was created from 38 samples with different oil content (Fig.1). Calibration model is strongly dependent on the accuracy of the primary extraction reference data and any inherent error of the primary extraction determinations would negatively impact on the performance of the FT-NIR calibration model [8].
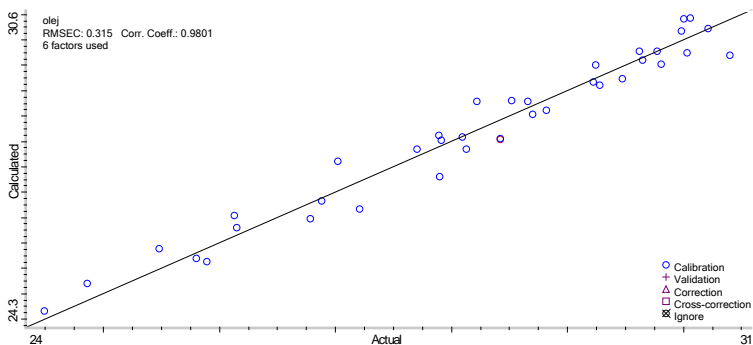

Fig. 1 The calibration model of 38 samples

The range of the calibration model was chosen with using Statistical Spectra. The range 11 446 – 4 000 cm^{-1} was chosen because the spectra were not showing any deviation peeks.

The values for the calibration model were RMSEC-0.315 (Fig.1), RMSEP-0.358 (Fig.2), RMSECV-0.45700, R-0.9801 (Fig.1).
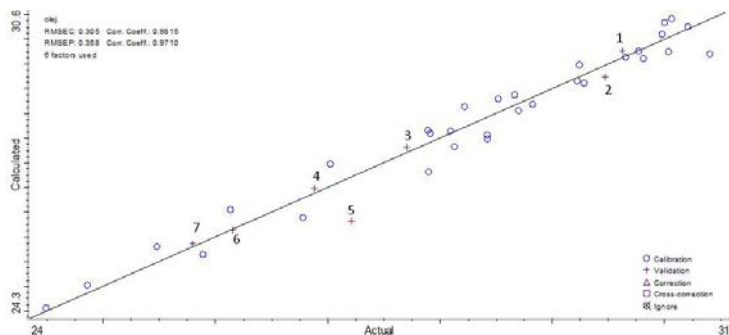
Fig. 2 The calibration model of 31 samples with validation of 7 validation samples

Table 1 The percentage of oil in the sample determined by the extraction and calculated using the created calibration model with tested validation standards.

sample	actual % of oil	calculated % of oil	S Standard deviation
1	29.62	29.77	0.11
2	29.47	29.24	0.16
3	27.7	27.81	0.08
4	26.88	26.98	0.07
5	27.21	26.32	0.63
6	26.15	26.13	0.01
7	25.8	25.87	0.05

The standard deviation of the sample 5 was higher than the others, because some of the samples were not homogenized.
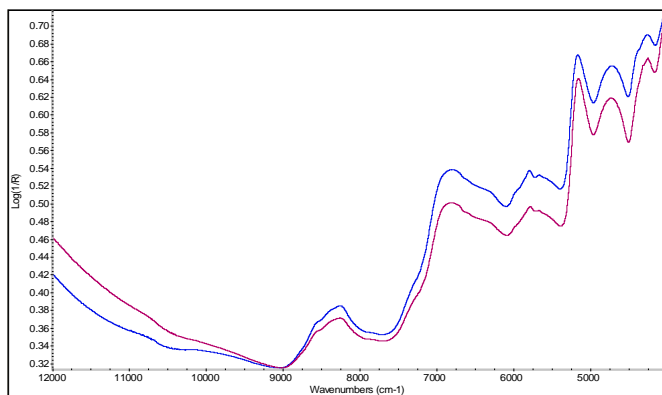

Fig. 3 The record of the sample with the highest and lowest oil content

The blue curve (upper) is lowest oil content in the sample (24.49%) and the red curve (lower) is the highest oil content in the sample (30.40%).

CONCLUSIONS

The calibration model for determination of the oil content in whole Milk Thistle seeds was created using the FT-NIR method. By measuring and comparing the validation samples it was confirmed that we have a robust model that can be further modified and extended to other samples and the measurement could be even better with reduced variations in measurements.

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