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# High-throughput screening for protein content in blue, yellow and white lupins

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## Abstract

Different lupin species (blue lupins, *L. angustifolius*; yellow lupins, *L. luteus*; white lupins, *L. albus)* were analyzed for the protein content in the growing seasons 2010 and 2012. As a reference method for the determination of the protein content, the Kjeldahl method was used. Based on these data a high-throughput screening method for whole lupin seeds was developed using NIRS analyses on a Bruker Multi Purpose Analyzer. The best calibration, cross validation, and prediction of independent samples was observed for whole seeds of blue lupins (RMSECV=0.924, $R^2$=0.82) followed by yellow (RMSECV=1.05, $R^2$=0.67) and white lupin seeds (RMSECV=1.08, $R^2$=0.65).

*Keywords*

*Lupinus albus*, *Lupinus angustifolius*, *Lupinus luteus*, NIRS, quality, whole seeds

## Introduction

The high protein content in lupin seeds is important for animal feed as well as for human nutrition. Yellow and white lupins have a higher protein content than blue lupins, but still need to be improved with respect to agronomic performance (WEHLING et al. 2012). For all lupin species a high protein content is an important breeding goal. Up to now NIRS methods using ground whole meal (JANSEN et al. 2006, BERK et al. 2008) and whole seeds are described (JANSEN et al. 2006, JANSEN and KUHLMANN 2007). To be able to screen efficiently for the protein content within lupin breeding programmes, NIRS methods facilitating a precise determination of the protein content in different lupin species have to be developed.

## Material and methods

### *Plant material*

Seeds of two varieties ('Boruta' and 'Haags Blaue') and six breeding lines of blue lupin grown at the location Bocksee in four replications, sixteen breeding lines grown in Steinach in two replications and eight breeding lines grown in Groß Lüsewitz in two replications were analysed in 2010. Seeds of twelve varieties ('Taper', 'Borena', 'Boresa', 'Borsaja', 'Juno', 'Pootallong', 'Popiel', 'Parys', 'Wasch', 'Bosch', 'Amulett' and 'Piast') and four breeding lines of yellow lupin grown in three locations (Groß Lüsewitz, Steinach and Triesdorf) in three replications were analyzed in 2012. The seeds of blue and yellow lupins were provided by Saatzucht Steinach.

Seeds of two varieties ('Àmiga' and 'Feodora') and fifteen breeding lines of white lupin grown at three locations (Groß Lüsewitz, Steinach and Triesdorf) in three replications were analyzed in 2012. Seeds of white lupins were provided by Landwirtschaftliche Lehranstalten, Pflanzenbau und Versuchswesen Triesdorf.

## Methods

The chemical determination of raw protein content was conducted using the Kjeldahl method (KJELDAHL 1883). For the development of a high-throughput screening method for the determination of the protein content in different lupin species, a NIRS method was used. Seed samples were scanned in duplicate with a Bruker-Fourier-Transform-Spectrometer Multi Purpose Analyzer (MPA, Bruker Optik GmbH, Ettlingen, Germany) equipped with the OPUS software package (Version 6.5). Absorption spectra were recorded between 800 and 2500 nm, using 32 scans per sample. To develop calibration models, the OPUS Quant multivariate calibration software of Bruker Optik incorporating Partial Least Squares (PLS) regressions was used to develop calibration models.

## Results and discussion

The mean value and the variation in the protein content of different lupin species is described by JANSEN and BALKO (2012). In the present study yellow lupins had the highest seed protein content followed by white lupins and blue lupins. The variation and mean values of the investigated lupin seeds using the Kjeldahl-method is shown in *Table 1*. Assuming moisture content of the seeds of approximately 10% the values are similar to those already reported. The protein content of the investigated blue, white and yellow lupin seeds varied between 27 and 40% (mean: 33%), 32 and 43% (mean: 39%) and 38 and 48% (mean: 43%), respectively, in whole dry seeds.

*Table 1:* **Protein content (% in DM) of different lupin species**

| Species | *n* | Mean | Variation |
|---|---|---|---|
| *L. angustifolius* | 79 | 33 | 27-40 |
| *L. albus* | 147 | 39 | 32-43 |
| *L. luteus* | 144 | 43 | 38-48 |

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*Table 2:* **Results of calibration and validation data of protein prognosis on different whole lupin seeds**

| Species | Parameter | Calibration | Validation | Test-Set-Validation |
|---|---|---|---|---|
| *L. angustifolius* | $R^2$ | 0.93 | 0.82 | 0.76 |
| | RPD | 3.86 | 2.36 | 2.08 |
| | Error | RMSEE 0.585 | RMSECV 0.924 | RMSEP 1.14 |
| *L. luteus* | $R^2$ | 0.70 | 0.67 | 0.63 |
| | RPD | 1.83 | 1.73 | 1.65 |
| | Error | RMSEE 1.01 | RMSECV 1.05 | RMSEP 1.13 |
| *L. albus* | $R^2$ | 0.76 | 0.65 | 0.57 |
| | RPD | 2.04 | 1.68 | 1.52 |
| | Error | RMSEE 0.908 | RMSECV 1.08 | RMSEP 1.15 |

$R^2$, coefficient of determination; RMSEE, root mean square error of calibration; RMSECV, root mean square error of cross validation; RMSEP, root mean square error of prediction; RPD, ratio of sample standard deviation to standard error of prediction

An effective NIRS-method for determining the protein content in *L. angustifolius* with high precision was described by JANSEN and KUHLMANN (2007) using single seeds as a matrix (transmission measurements, Infratec® 1255, Fa. Foss). Another method was presented by JANSEN et al. (2006) using whole meal and whole seeds (reflection measurements, NIRS™ 5000, Fa. Foss). Reflection measurements on whole seeds were also applied in this paper. Data concerning quality of the calibration and validation experiments are presented in *Table 2*.

Best results were obtained for the prediction of the protein content in whole seeds of blue lupins, which RMSECV=0.924 and $R^2$=0.82. The use of NIRS-methods for the determination of protein content is well known in other species. An overview about applications in e.g. wheat quality control (breeding, production, trade, milling) is given by POJIĆ et al. (2012). NIRS methods have been accepted as standard methods by the ISA, AACC, AOAC and ICC. A calibration for predicting protein in single kernel of barley (Validation $R^2$=0.84) was reported by FOX et al. (2011). MÍKA et al. (2003) described the prediction of the protein content in whole rape seed (Validation $R^2$=0.83, VDLUFA network).

In experiments to predict the digestible protein of lupin kernel meal GLENNCROSS et al. (2008) determined a RMSECV=2.7% and $R^2$=0.47. In comparison to these experiments it may be concluded that the NIRS calibration developed in this study can be efficiently used for screening the protein content in lupin breeding programs.

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## References

BERK A, BRAMM A, BÖHM H, AULRICH A, RÜHL G, 2008: The nutritive value of lupins in sole cropping systems and mixed intercropping with spring cereals for grain production. In: PALTA JA, BERGER JB (eds), Lupins for health and wealth, Proc 12th Int Lupin Conf, 14-18 Sep, Fremantle, Western Australia, 66-80. Int Lupin Ass, Canterbury, New Zealand.

FOX GP, KELLY A, SWEENEY N, HOCORFT DM, 2011: Development of a single kernel NIR barley protein calibration and assessment of variation in protein on grain quality. J Inst Brew 117, 582-586.

GLENNCROSS B, HAWKINS W, BURRIDGE P, EVANS D, RUTHERFORD N, MCCAFFERTY P, DODS K, SIPSAS S, 2008: Using near-infrared reflectance spectroscopy (NIRS) to predict the digestible protein and energy value of lupin kernel meals when fed to rainbow trout, *Oncorhynchus mykiss*. In: PALTA JA, BERGER JB (eds), Lupins for health and wealth, Proc 12th Int Lupin Conf, 14-18 Sep, Fremantle, Western Australia, pp. 108-114. Int Lupin Ass, Canterbury, New Zealand.

JANSEN G, BALKO C, 2012: Leguminosen unter Stress - Stabilität von Ertrag und Qualität. Das Blatt 1 (4/2012), 48-51.

JANSEN G, JÜRGENS HU, FLAMME W, 2006: Züchterische Bearbeitung von Süßlupinen für den ökologischen Landbau - Qualitätsuntersuchungen im Hinblick auf Futtereignung. FKZ: 03OE355. BÖL-Bericht-ID 11087. [http://orgprints.org/11087/].

JANSEN G, KUHLMANN J, 2007: Proteinuntersuchungen in Einzelsamen von Lupinen zur züchterischen Erhöhung des Eiweißgehaltes. In: ZIKELI S, CLAUPEIN W, DABBERT S, KAUFMANN B, MÜLLER T, VALLE ZÁRATE A (Hrsg), Zwischen Tradition und Globalisierung - Beiträge zur 9. Wissenschaftstagung Ökologischer Landbau, 20.-23. März, Stuttgart-Hohenheim, Band 1, 263-266. Verlag Dr. Köster, Berlin [http://orgprints.org/9084/].

KJELDAHL J, 1883: Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. Z Anal Chem 22, 366-382.

MÍKA V, TILLMANN P, KOPRNA R, NERUŠIL P, KUČERA V, 2003: Fast prediction of quality parameters in whole seeds of oilseed rape (*Brassica napus* L.). Plant Soil Env 49, 141-145.

POJIĆ M, MASTILOVIĆ J, MAJCEN N, 2012: The application of near infrared spectroscopy in wheat quality control. In: Theophanides T (ed), Infrared spectroscopy - Life and biomedical sciences, 167-184. InTech, Rijeka, Croatia.

WEHLING P, RUGE-WEHLING B, RUDLOFF E, JANSEN G, BALKO C, 2012: Auf dem Weg zu einer nachhaltigen Tierernährung. Forschungsreport Ernährung, Landwirtschaft, Verbraucherschutz 1/2012, 11-15.