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RESEARCH ARTICLE

Near-Infrared Determination of Total Soluble Nitrogen and Betaine in Sugar Beet

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Abstract The total soluble nitrogen (TSN) content in sugar beets strongly hinders sugar extraction. Traditionally, the amount of TSN is not measured directly, but inferred from the amount of amino nitrogen (~30% of the TSN) in the beet. Betaine, the other main TSN component, accounts approximately for another 30%. Betaine also interferes with sugar crystallization, and it is a highly interesting metabolite in pharmaceutics and agronomics. The aim of this study was to develop non-invasive near-infrared (NIR) applications to measure the TSN and betaine content in beets in a fast and reliable way. Sugar beets were harvested for up to five harvest periods, and pulp samples were measured with a NIR system. Calibration models reached correlations (R) between laboratory and predicted values of 0.823 for TSN and 0.947 for betaine, respectively. The prediction of independent validation sets showed also high correlation coefficients for both TSN ($R = 0.756$) and betaine ($R = 0.837$). These NIR applications could be very helpful in the assessment of beet quality in breeding programs and industrial processes.

Keywords Near-infrared spectroscopy · Total soluble nitrogen · Betaine · Sugar beet

Introduction

The amount of sugar that can be extracted from a specific sugar beet variety is the main character that accounts for a high beet quality and therefore the most relevant trait for the sugar industry, beet growers, and beet breeders. There are several components in the beet that have a negative influence on the sugar extraction, mainly the total soluble nitrogen (TSN), sodium, and potassium content (van der Poel et al. 1998). Sodium and potassium are routinely measured by the sugar industry and breeders to assess beet quality. The determination of TSN is on the other hand difficult to introduce in processes involving a big number of samples due to high laboratory cost and long sample processing. TSN levels in beets have been highly correlated to levels of free amino acids or, in short, amino nitrogen (amino N) (Schiweck et al. 1994; Hoffmann 2005, 2006). The determination of amino N is precise, fast, and has a low cost. As a result, the only fraction of the TSN analysed at the moment to assess beet quality is the amino N component. However, the amino N fraction constitutes on average only about 30% of the TSN content. Betaine is the other main TSN component, accounting for approximately another 30% (Burba et al. 1984; Hoffmann 2006). As with all TSN constituents, high betaine levels in sugar beet roots are undesirable because it interferes with sucrose crystallization (Hoffmann and Märländer 2005). Physiologically, betaine helps to maintain the osmotic balance in the cell and it is a source of methyl groups for use in many biochemical pathways (Hanson and Wyse 1982; Craig 2004). These traits make of betaine a highly valuable metabolite with a broad spectrum of applications in agronomics and pharmaceutics (Craig 2004; Mäkelä 2004; Day and Kempson 2016).

It has been shown that a low content in amino N in the beet is compensated with a higher content in betaine (Hoffmann and Märländer 2005; Hoffmann 2005). Therefore, beets with a low content of amino N could be too well rated concerning the total content of nitrogen or vice versa, since betaine is not included in the evaluation. This

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problem is well known, and the suggestion to improve the quality assessment by adding TSN and/or betaine measurements to the standard analyses has been done already by several authors (Burba and Schiweck 1993; Beiss 1994; Burba 1996, Burba et al. 2001; Hoffmann and Märländer 2005; Hoffmann 2006; Dutton and Huijbregts 2006).

Since the late 1980s, near-infrared (NIR) spectroscopy technology has been steadily spreading in agriculture (Givens and Deaville 1999). Well-established NIR applications are for instance the measurement of protein content in wheat and hardness in maize (Fox and Manley 2009; Williams 2010; Pojić et al. 2012). In the sugar beet sector in particular, NIR spectroscopy is increasingly being used to measure the sucrose content in beets (Heppner et al. 2000; Roggo et al. 2004). Well-known advantages of this technology are to be a non-destructive method, cost efficient, a small amount of sample is required, high precision, a very quick measurement, easy sample preparation (without added chemicals), and it is possible to determine several substances at once. Altogether, these factors allow a higher number of analysed samples, and at much lower costs, than traditional laboratory methods.

Materials and Methods

Sampling: NIR measurement was integrated in the harvesters, and measurements were arranged in parallel to the harvest process. The field trials consisted of an alpha lattice design layout with three row plots of 9 m^2. All sugar beets in a plot were harvested, topped, washed, and sawn into a very fine pulp. This pulp was well mixed to obtain a homogeneous and representative pulp sample for each plot. The pulp was spread in 7-mm-thick plates that were immediately measured by NIR. Spectra were measured under reflection with the spectrometer PSS 1720 (Polytec GmbH, Waldbronn, Germany) every 2 nm, in the spectral range 850–1650 nm. One NIR measurement takes 3.2 s. After measurement, the plates were covered and stored at −20 °C for further analytical procedures.

Total soluble nitrogen determination: 716 samples were collected over five harvest periods: 2009 (69 samples), 2010 (189 samples), 2011 (222 samples), 2014 (120 samples), and 2015 (116 samples) and sent for TSN content determination to the Institute for Feed Analysis (LUFA Nord-West) in Oldenburg (Germany). The Kjeldahl method (VDLUFA Bd.III 4.1.1) was used to determine the percentage of TSN in fresh pulp. Samples were harvested at 20 different sites in Germany and France with 17 and 3 sites, respectively.

Betaine determination: 375 samples were collected over four harvest periods: 2012 (140 samples), 2013 (33 samples), 2014 (92 samples), and 2015 (110 samples) and sent for analysis to Nordzucker AG in Braunschweig (Germany). HPLC was used to determine the percentage of betaine in fresh pulp (ICUMSA Method GS 4-22 2002). Samples were harvested at 15 different sites in Germany and France with 13 sites and 2 sites, respectively.

The genotypes used for TSN and betaine analyses were chosen randomly in every harvest period and differ from year to year. Therefore, the samples in this study are to be considered as individual samples and not as replicas.

Amino N determination: All above-mentioned samples collected for TSN and/or betaine analyses, 925 samples in total, were also analysed for amino N content at Strube Research GmbH & Co. KG by using the fluorometric OPT (O-Phthalaldehyde) method.

Chemometrical analyses: Calibration models were developed using the SensoLogic Calibration Workshop v.2.10 and SensoLogic Calibration Wizard v.2.0 software packages (SensoLogic GmbH, Norderstedt, Germany). Data were separated into a calibration and a validation set. One in every five samples was selected to create an independent and representative validation set. The remaining samples constituted the calibration set. Calibrations were calculated with means of partial least squares regression (PLSR) proceeded by several spectroscopic data pretreatments. Combinations of first derivative, second derivative, normalization, and standard normal variate were used to optimize calibrations. The performances of the different models obtained during the calibration phase were determined from cross-validation as an internal validation method. In addition, the external validation set was used to test the prediction capacity of the models. The best calibration models for each parameter were selected based on a low root-mean-square error of cross-validation (RMSECV), low bias, increasing correlation coefficient (R) in calibration and prediction, and decreasing standard error of prediction (SEP) and root-mean-square error of prediction (RMSEP).

Statistical analyses: To further evaluate the performance of the calibration models, heritability (h^2) analyses were carried out with TSN and betaine values predicted with the optimized calibrations. None of the samples included in the NIR calibrations were used for h^2 calculations. As a means of comparison, h^2 was also calculated with laboratory amino N values determined for the same sets of samples. Heritability was calculated according to the formula of Hallauer and Miranda (1981):

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gl}^2}{l} + \frac{\sigma_e^2}{rl}}$$

where σ_g^2 is the genotypic variance σ_{gl}^2 the genotype-location interaction variance, and σ_e^2 s the error variance. The number of replications is r and the number of locations is l.

Heritability calculations were performed for three individual sites from the harvest period 2015, in which all harvested samples were also measured with NIR. Since each location was analysed separately, the formula of heritability reduces to repeatability within a site. Data sets used for h^2 calculations consisted of: three trials (266 samples) for a site near Terny–Sorny (France), 12 trials (1240 samples) for a site near Pithiviers (France), and eight trials (606 samples) for a site near Soest (Germany). Laboratory values for TSN and betaine were not available for these samples; thus, a direct comparison between NIR estimated and laboratory values could not be calculated. The variance components were calculated using a mixed model, $y = \mu : g + r + b$ which consisted of random effects including genotype (g), replication (r), and block (b) (Piepho et al. 2003). Data were also tested for normal distribution and variance homogeneity. Statistical analyses were performed with GenStat v.14 (VSN International 2011).

Results

The complete spectral range measured (850–1650 nm) was used for calibration development. For TSN, the best spectrometric pre-treatment consisted of a second derivative followed by a standard normal variate transformation. For betaine, the best results were obtained by applying a first derivative followed by a standard normal variate. In the optimized calibration model for TSN, the Pearson correlation coefficient reached $R = 0.823$, while for betaine, the correlation coefficient reached $R = 0.947$ (Fig. 1). The prediction of the independent validation data sets showed that the predicted NIR values were adjusting well to the laboratory values for both parameters. Calculated correlation coefficients were 0.756 for TSN and 0.837 for betaine, respectively. The parameters indicating predictive ability, SEP and RMSEP, were equal up to the third decimal for both models, TSN and betaine. The SEP squared is approximately equal to the RMSEP squared minus the bias squared. Therefore, like in the present data, if the bias is low, the values for RMSEP and SEP will be similar. The bias obtained for the prediction of both validation sets were 0.001 for TSN and −0.001 for betaine, indicating a low systematic difference between the values obtained in the laboratory and the values obtained with the NIR prediction. The main characteristics from both calibration and prediction data sets, as well as the main descriptive parameters for calibration models and predictions, are summarized in Table 1.

A comparison among TSN, betaine, and amino N was also carried out by considering only the obtained laboratory values (Table 2). The TSN content in the 716 samples

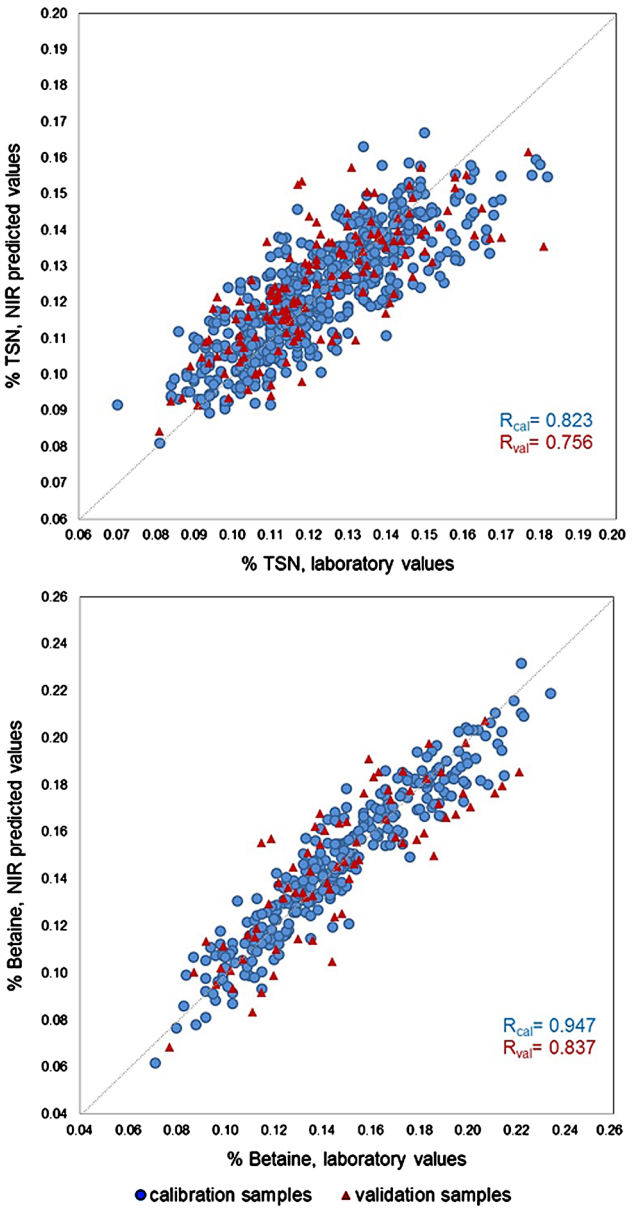

Fig. 1 Calibration models for TSN ($n = 572$) and betaine ($n = 300$) in sugar beet pulp. Laboratory values versus NIR-predicted values are indicated. The prediction of the validation samples is also shown ($n = 144$ for TSN and $n = 75$ for betaine), as well as correlation coefficients for calibration and validation

analysed ranged from 0.070 to 0.182% in fresh pulp. The percent of betaine in the 375 samples analysed ranged from 0.071 to 0.234% in fresh pulp. One hundred and sixty-six samples comprising two harvesting periods (2014 with 56 samples and 2015 with 110 samples) could be analysed in common for TSN and betaine. The amino N content for all 925 samples ranged from 0.27 to 2.66 mM per 100 g^{-1} pulp. Betaine levels presented a low correlation with amino N levels ($R = 0.442$), in contrast to betaine and TSN levels, where a high correlation was observed ($R = 0.799$).

Table 1 Descriptive characteristics of NIR calibration models for TSN and betaine in sugar beet

	%TSN	%Betaine
Calibration set		
Samples	572	300
Years	2009, 2010, 2011, 2014, 2015	2012, 2013, 2014, 2015
Property range	0.070–0.182	0.071–0.234
Calibration parameters		
R laboratory/ predicted	0.823	0.947
SEC	0.010	0.010
RMSECV	0.013	0.014
PLSR variables	11	15
Validation set		
Samples	144	75
Years	2009, 2010, 2011, 2014, 2015	2012, 2013, 2014, 2015
Property range	0.081–0.181	0.077–0.221
Validation parameters		
Bias	0.001	−0.001
SEP	0.013	0.018
RMSEP	0.013	0.018
R laboratory/ predicted	0.756	0.837

Units for TSN and betaine: % from fresh pulp

TSN total soluble nitrogen, *PLSR* partial least squares regression; *SEC* standard error of calibration, *RMSECV* root-mean-square error of cross-validation, *SEP* standard error of prediction, *RMSEP* root-mean-square error of prediction

A moderate correlation was found between amino N and TSN levels ($R = 0.593$). When looking at the data on a yearly basis, higher correlations between TSN and amino N were observed for all periods (R ranged from 0.758 to 0.929) except for 2011 ($R = 0.522$). All correlations were found to be highly significant.

Concerning the heritability analyses, in two of the three tested sites, betaine NIR-predicted values showed better h^2 results than TSN NIR-predicted values and amino N laboratory values. The h^2 values for amino N were higher than for TSN or betaine only in the Soest site (Table 3).

Discussion

Selection against components that impair an optimal sugar extraction is of extreme importance. Due to analytical limitations, however, the determination of some of these factors such as TSN or betaine is not established as a standard quality screen so far. The hereby presented NIR models show a good adjustment between NIR and laboratory values in calibration as well as in prediction, and thus demonstrate that an accurate NIR determination of TSN and betaine is possible. The measurement of both parameters takes less than 4 s, and it could be easily incorporated in sugar beet pulp analyses for large amount of samples. Heritability calculations supported a good prediction result, since h^2 for TSN- and betaine-predicted values were in the same order of magnitude as h^2 for amino N laboratory values. NIR applications performed with the same repeatability within a location as standard laboratory measurements.

A first important step in the sugar beet nitrogen measurement with NIR was done by Burba et al. (2001), when they successfully calculated a NIR calibration for TSN. They obtained a coefficient of correlation between laboratory and NIR values of 0.985. The calculation of SEC and SEP was dismissed due to the small number of samples available. Only a single harvest period, 79 samples for calibration and 12 samples for validation were available in that study. The introduction of different harvesting years in agricultural NIR applications is a very important factor in the process of calibration optimization (Igne et al. 2007). In sugar beet breeding, it is well known that the year effect is by far the most important factor affecting yield parameters (Freckleton et al. 1999; Stockfisch et al. 2002; Kenter et al. 2006). The composition in the beet is influenced by genotype, but also by environmental factors including soil preparation, fertilizer, crop rotation, presence of diseases, amount of rain, and temperature, which can vary significantly from year to year. The results achieved by Burba et al. (2001) indicated that the detection of TSN with NIR was feasible within a given year. As they also point out, however, their model as such had no predictive value in practice, because year effects were not introduced in the calibration. Besides genotype and environment as such, genotype by environment interaction (GEI) does occur (Hoffmann et al. 2009; Mackay et al. 2011; Liebe and Varrelmann 2016). Variation in the beets due to GEI effects could decrease the prediction accuracy if these factors are not enclosed in the calibration models. In order to decrease SEP to a minimum, the NIR calibration needs to encompass several sites and several harvesting periods.

Considering laboratory analyses, the correlation between betaine and TSN observed in the present data ($R = 0.799$) is similar to the correlation reported by Burba et al. (2001) ($R = 0.752$). The moderate ($R = 0.593$) but highly significant correlation obtained between laboratory values for amino N and TSN was close to the correlation reported by Burba (1996) ($R = 0.691$), but unexpected considering previous studies which found correlations at the 0.9 level (Schiweck et al. 1994; Hoffmann 2005, 2006). In the present data, a 0.9 correlation is only reached for the harvest period 2015. This might lead to consider that a high TSN-amino N correlation cannot be assumed under every circumstance, since environment has clearly a strong

Table 2 Correlation coefficient (R) among laboratory values for TSN, betaine, and amino N

	Amino N	Betaine	TSN	Samples	Years
All available samples					
Amino N		0.442***		375	2012, 2013, 2014, 2015
Betaine			0.799***	166	2014, 2015
TSN	0.593***			716	2009, 2010, 2011, 2014, 2015
Divided in years					
Amino N		0.451***		140	2012
Amino N		0.512**		33	2013
Amino N		0.614***		92	2014
Amino N		0.730***		110	2015
Betaine			0.722***	56	2014
Betaine			0.822***	110	2015
TSN	0.758***			69	2009
TSN	0.827***			189	2010
TSN	0.522***			222	2011
TSN	0.760***			120	2014
TSN	0.929***			116	2015

*** Significantly different at: $P < 0.001$

** Significantly different at: $P < 0.01$

Table 3 Heritability coefficients for three sites from harvest period 2015

Site	TSN	Betaine	Amino N
Soest	0.48	0.49	0.80
Pithiviers	0.60	0.79	0.56
Terny-Sorny	0.57	0.67	0.63

NIR-predicted values were taken for the TSN and betaine calculations. Laboratory values were used for the amino N calculations

impact on the nitrogen components in beet. Specifically, the betaine content has been reported to be affected by irrigation, year, site, variety, fertilizers, and topping (Smed et al. 1996). Additionally, it has been shown that betaine and amino N react markedly differently to environment effects (Hoffmann et al. 2009). For instance, under increased nitrogen supply in soil, the amino N fraction in the beet has been described to increase, while the betaine fraction decreases (Hoffmann 2005). However, contradictory results have been presented as well, with an increase in betaine in beet following nitrogen supply (Beiss 1994; Burba 1996). Levels of betaine have been reported to rise under stress situations such as drought, towards the end of the growing period, at a low plant density, and to be higher in beet crowns (Hanson and Wyse 1982; Beiss 1994; Gzik 1996; Shaw et al. 2002; Hoffmann 2006; Choluj et al. 2008). This last point might be especially relevant when beets are being harvested only defoliated and not topped.

Betaine levels are omitted nowadays by the standard quality analyses. Betaine is of interest considering its role in hindering sugar extraction, and it is also a highly valuable compound with an increasing range of agronomical, nutritional, and medical uses. The world betaine market seems to be expanding, and it is projected to reach USD 3.89 billion by 2020 (www.marketsandmarkets.com 2015). The screening and selection of varieties with high betaine content could become an additional economical factor to be considered by beet farmers and sugar industry. Paramount to explore this possibility would be the incorporation of a betaine measurement in routine quality analyses. Interestingly, it has been shown that in a range of environments, the genotype effect is bigger for betaine (8.5%) than for amino N (3.2%) (Hoffmann et al. 2009). This could be due to an increased breeding pressure against the amino N levels in the beet, while the betaine has not been, or could not have been, selected for so far.

Growers, sugar industry, and breeding programs might benefit from a direct measurement of TSN and betaine in a fast, low-cost, and accurate manner. NIR technology could make this possible. Further research might be required to better understand the GEI effects in the relation TSN–betaine–amino N. Increasing the number of samples, locations, and harvest periods might improve further the performance of the calibration models hereby presented. However, the results of this study clearly indicate that the measurement of TSN and betaine is possible with NIR in a simple and reliable way. The introduction of such NIR

applications in routine analyses could be of great help for the quality assessment of sugar beet varieties.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Beiss, U. 1994. Betain content in sugar beet. *Zuckerindustrie* 119(2): 112–117.

Burba, M., U. Nitzschke, and R. Ritterbusch. 1984. Die N-Assimilation der Pflanze unter besonderer Berücksichtigung der Zuckerrübe. *Zuckerindustrie* 109(7): 613–629.

Burba, M., and H. Schiewek. 1993. The nonsugar and ion balance of thick juice as bases for a quality assessment of sugar beets: Part 2. *Zuckerindustrie* 118(12): 921–936.

Burba, M. 1996. Harmful nitrogen as a criterion of beet quality. *Zuckerindustrie* 121(3): 165–173.

Burba, M., T. Huijbregts, and E. Hilscher. 2001. On the determination ot total soluble nitrogen in sugarbeet by Near Infrared Spectrometry. *NIRS. Zuckerindustrie* 126(5): 367–375.

Choluj, D., R. Karwowska, A. Ciszewska, and M. Jasińska. 2008. Influence of long-term drought stress on osmolyte accumulation in sugar beet (*Beta vulgaris* L.) plants. *Acta Physiologiae Plantarum* 30: 679–687.

Craig, S. 2004. Betaine in human nutrition. *American Journal of Clinical Nutrition* 80: 539–549.

Day, C.R., and S.A. Kempson. 2016. Betaine chemistry, roles, and potential use in liver disease. *Biochimica et Biophysica Acta* 1860(6): 1098–1106.

Dutton, J., and T. Huijbregts. 2006. Root Quality and Processing. In *Sugar Beet*, ed. A.P. Draycott, 409–442. Oxford: Blackwell Publishing Ltd.

Fox, G., and M. Manley. 2009. Hardness methods for testing maize kernels. *Journal of Agricultural and Food Chemistry* 57(13): 5647–5657.

Freckleton, R.P., A.R. Watkinson, D.J. Webb, and T.H. Thomas. 1999. Yield of sugar beet in relation to weather and nutrients. *Agricultural and Forest Meteorology* 93(1): 39–51.

Givens, D.I., and E.R. Deaville. 1999. The current and future role of near infrared reflectance spectroscopy in animal nutrition: a review. *Australian Journal of Agricultural Research* 50: 1131–1145.

Gzik, A. 1996. Accumulation of proline and pattern of α-amino acids in sugar beet plants in response to osmotic, water and salt stress. *Environmental and Experimental Botany* 36: 29–38.

Hallauer, A.R., and J.B. Miranda. 1981. *Quantitative genetics in maize breeding*. Ames: Iowa State University Press.

Hanson, A.D., and R. Wyse. 1982. Biosynthesis, Translocation, and Accumulation of Betaine in Sugar Beet and Its Progenitors in Relation to Salinity. *Plant Physiology* 70(4): 1191–1198.

Heppner, S., K. Thielecke, K. Buchholz, and D. Wullbrandt. 2000. Potential applications of NIR spectrometry in the sugar industry. *Zuckerindustrie* 125(5): 325–330.

Hoffmann, C. 2005. Changes in N composition of sugar beet varieties in response to increasing N supply. *Journal of Agronomy and Crop Science* 191: 138–145.

Hoffmann, C., and B. Märländer. 2005. Composition of harmful nitrogen in sugar beet (*Beta vulgaris L.*) - amino acids, betaine, nitrate—as affected by genotype and environment. *European Journal of Agronomy* 22: 255–265.

Hoffmann, C., 2006. *Zuckerrüben als Rohstoff*. Göttingen, Postdoctoral Thesis.

Hoffmann, C., T. Huijbregts, N. van Swaaij, and R. Jansen. 2009. Impact of different environments in Europe on yield and quality of sugar beet genotypes. *European Journal of Agronomy* 30: 17–26.

Igne, B., L.R. Gibson, G.R. Rippke, and C.R. Hurburgh. 2007. Influence of Yearly Variability of Agricultural Products on Calibration Process: A Triticale Example. *Cereal Chemistry Journal* 84(6): 576–581.

Kenter, C., C. Hoffmann, and B. Märländer. 2006. Effects of weather variables on sugar beet yield development (*Beta vulgaris* L.). *European Journal of Agronomy* 24(1): 62–69.

Liebe, S., and M. Varrelmann. 2016. Effect of Environment and Sugar Beet Genotype on Root Rot Development and Pathogen Profile During Storage. *Phytopathology* 106(1): 65–75.

Mackay, I., A. Horwell, J. Garner, J. White, J. McKee, and H. Philpott. 2011. Reanalyses of the historical series of UK variety trials to quantify the contributions of genetic and environmental factors to trends and variability in yield over time. *Theoretical and Applied Genetics* 122(1): 225–238.

Mäkelä, P. 2004. Agro-industrial uses of glycinebetaine. *Sugar Tech* 6(4): 207–212.

Marketandmarkets. 2015. Betaine Market by Type (Synthetic Betaine and Natural Betaine), Form (Betaine Anhydrous, Cocamidopropyl Betaine, Betaine Monohydrate), Application (Food & Beverages, Animal Feed, Cosmetics, Detergents), and by Geography - Global Forecast to 2020. http://www.marketsandmarkets.com.

Piepho, H.P., A. Büchse, and K. Emrich. 2003. A Hitchhikers Guide to Mixed Models for randomized experiments. *Journal of Agronomy and Crop Science* 189: 310–322.

Pojić, M., J. Mastilović, and N. Majcen. 2012. The Application of Near Infrared Spectroscopy in Wheat Quality Control. In *Infrared Spectroscopy - Life and Biomedical Sciences*, ed. T. Theophile, 167–184. Rijeka: InTech.

van der Poel, P.W., H. Schiweck, and T. Schwatz. 1998. *Sugar technology beet and cane sugar manufacture*. Berlin: Dr. Albert Bartens KG.

Roggo, Y., L. Duponchel, and J.-P. Huvenne. 2004. Quality evaluation of sugar beet (*Beta vulgaris*) by near-infrared spectroscopy. *Journal of Agricultural and Food Chemistry* 52(5): 1055–1061.

Schiweck, H., G. Kozianowski, J. Anderlei, and M. Burba. 1994. Calculation of the nonsugar mass in thick juice from beta analyses - Proposal of a formula to assess the technical quality of sugar beets. *Zuckerindustrie* 119(4): 268–282.

Shaw, B., T.H. Thomas, and D.T. Cooke. 2002. Responses of sugar beet (*Beta vulgaris L.*) to drought and nutrient deficiency stress. *Plant Growth Regulation* 37: 77–83.

Smed, E., A.W.M. Huijbregts, D. Hadjiantoniou, and G. Vallini. 1996. Influence of agronomic factors on non conventional parameters of the internal quality of sugar beet. In *Proceedings of the 59th IIRB Congress* 385–389.

Stockfisch, N., H.-J. Koch, and B. Märländer. 2002. Effects of Weather on Growth and Dry Matter Yield of Sugar Beet. *Pflanzenbauwissenschaften* 6(2): 63–71.

VSN International. 2011. *GenStat for windows*, 14th ed. Hempstead: VSN International.

Williams, P. 2010. The analysis of wheat by near-infrared spectroscopy. In *Handbook of vibrational spectroscopy*, ed. E.C.Y. Li Chan, P.R. Griffiths, and J.M. Chalmers. New Jersey: John Wiley.