Stefan Meldau; Manfred Burba; Elke Hilscher

Past, present and future of sugar beet quality analysis: a mini review

Vergangenheit, Gegenwart und Zukunft der Qualitätsanalyse von Zuckerrüben: ein kurzer Abriss

Sugar beet quality analysis is an integral part of breeding and sugar beet processing and therefore contributes to the success of the beet sugar industry. This review summarizes the chronological development of important methods used for sugar beet quality analysis, with a special focus on the role that breeding companies, such as KWS, have played in establishing these methods. Finally, new technological advances are highlighted that provide possibilities to automate the analysis of quality parameters of fresh sugar beet outside of the laboratory.

Key words: sugar beet, *Beta vulgaris*, quality analysis, sugar content, sugar factory, processing, breeding

Die Qualitätsanalyse von Zuckerrüben ist integraler Bestandteil der Züchtung und Verarbeitung von Zuckerrüben und trägt somit zum Erfolg der Rübenzuckerindustrie bei. Dieser Überblick fasst die chronologische Entwicklung wichtiger Methoden zur Qualitätsanalyse von Zuckerrüben zusammen, wobei ein besonderer Schwerpunkt darauf liegt, welche Rolle Züchtungsunternehmen wie die KWS bei der Etablierung dieser Methoden gespielt haben. Schließlich werden neue technologische Fortschritte hervorgehoben, die Möglichkeiten bieten, die Analyse der Qualitätsparameter von frischen Zuckerrüben außerhalb des Labors zu automatisieren.

Schlagwörter: Zuckerrüben, *Beta vulgaris*, Qualitätsanalyse, Zuckergehalt, Zuckerfabrik, Verarbeitung, Züchtung

1 The uprise of the beet sugar industry in Europe in the 18th century

In 1747, *Andreas Sigismund Marggraf* revealed that beet (*Beta vulgaris*) contain the same sugar as cane (*Saccharum officinarum*) (*Marggraf*, 1747). His analysis of beet by alcoholic extraction and evaporation was based on *Marggraf's* pharmacological expertise. It took more than 50 years of breeding and technological improvements until sugar could be extracted out of beet on an industrial scale. This was achieved by one of *Marggraf's* students, *Franz Carl Achard*, in 1802, when he opened the first European sugar factory in Cunern (Konary, Poland) (*Achard*, 1809; *Müller*, 2002). Initially, the factory extracted 30 kg of sugar out of 1 t of beet, with a capacity of 5 t/d. *Achard* already realized that the sugar manufacture was being hampered by other components in the beet, but was unable to further specify the culprits (*Achard*, 1809).

Due to *Napoleon's* blockage of cane sugar imports, the young European sugar industry gained momentum in the early 19th century. When cane sugar imports were allowed again, the beet sugar industry could only be competitive if it was able to increase yield and sugar content of beet as well as processing efficiencies of the factories. Therefore, increasing the sugar content was a prime breeding target and analyzing sugar beet quality was crucial for improving beet processing in the factories.

2 From 1850: Breeding companies advance and quality analysis was developed

The initial methods for analyzing the sugar content in beet were indirect and not very precise. In the 19th century "start-up" sugar beet breeding companies such as KWS (founded in 1856, initially under Rabbethge & Giesecke OHG), or established breeding companies, such as Vilmorin in France, used simple density measurements to characterize their beet. This was done by weighing sugar beet in air and comparing the results to their mass in water (*Vilmorin*, 1859).

In 1862, *Matthias Rabbethge* Jr., the head of breeding and analysis at KWS was one of the first who used a polarimeter for measuring the sugar content in beet juice (*Meißner*, 2007). Since 1891, polarimeters were operated based on *Pellet's* flow-through method (*Pellet*, 1885), which helped to increase the throughput of measurements. The beet extraction method was changed in 1879 at KWS, where a hand-rasp was used to grind the beet into pulp (commonly called beet "brei" in German), followed by polarimetry of alcoholic extracts. Beet brei was later produced via a specific beet drilling device (Segmentreibe) developed by the Keil and Dolle company in 1887 (among other devices).

In 1888, *Clerc* described an aqueous brei extraction method. Lead acetate was used as a clarifying agent to precipitate proteins and colored pigments. This clarification of the extract increased the precision of the polarimetry measurements

(*Clerc*, 1888a; *Clerc*, 1888b; *Clerc*, 1888c). The precipitation by lead acetate solution described in 1904 (*Lippmann*, 1904) was then used as a standard procedure in the sugar beet breeding and processing industry.

It was clear that sugar content was not the only parameter that determines final sugar yield. Other beet constituents, such as potassium and sodium salts and organic nitrogen compounds, were identified to reduce sugar beet quality. In 1888, *Herzfeld* published that high nitrogen fertilization had a negative influence on sugar beet quality (*Herzfeld*, 1888). The importance of nitrogen compounds was later confirmed by *Andrlik* (*Andrlik*, 1904) and through extensive collaborations between KWS and the Berlin Sugar Institute (*Spengler* et al., 1933 and 1934). The so-called "harmful nitrogen" was indicated to be one of the main drivers reducing sugar beet quality. The α-amino-N that is measured in modern analyses is a part of the harmful nitrogen pool. However, methods for measuring nitrogen compounds in a reasonable throughput were unknown in the 19th century. Breeders at KWS analyzed the browning of brei (due to the conversion of tyrosine to melanin in air) as an indirect way to estimate nitrogen levels. In the 1860s in France, and later in Germany the soluble ash (soluble inorganic nonsugar components) was first analyzed via gravimetry (*Sostmann*, 1866).

To standardize the different methods used in the sugar industry, the ICUMSA (International Commission for Uniform Methods of Sugar Analysis) was founded in 1897 and still exists today as a global organization for sugar analysis methodology (ICUMSA).

3 From the 20th century to today

In the beginning of the 20th century, sugar beet breeders were able to increase sugar content in beet to 16–18%, yielding 4 t/ha sugar. The determination of soluble ash was improved by a newly developed conductometric analysis in the 1920s (*Tödt*, 1925), a methodology that was further improved in 1970 (*Burba*, 1970a). The measurement of sodium and potassium in sugar beet extracts via flame photometry was introduced in the 1960s and allowed the calculation of sugar loss to molasses (later called the standard molasses loss, SML) via various calculations (*Burba*, 1970b; *Burba*, 1971).

Colorimetric detection methods for amino acids and amides (*Friedl*, 1911) and especially the photometric copper-complex method ("Blauzahl", blue number method) allowed a quick and reliable analysis of soluble nitrogen-containing organic compounds with a reasonable throughput (*Stanek*, 1934). These methods were further improved and integrated into automated sugar beet quality laboratories in the 1960s and 1970s (*Burba* and *Lurz*, 1972b; *Reggelin*, 1965).

A major advance was the replacement of the toxic lead acetate by aluminum sulfate combined with the fluorometric determination of α-amino-N in brei extracts, both developed by KWS (*Burba*, 1996; *Burba* and *Lurz*, 1972b; *Burba* and *Georgi*, 1975 and 1976). Fluorometry is still a commonly used method in sugar beet quality laboratories and many formulas for calculating sugar beet quality consider α-amino-N because of its relatively high correlation with the total soluble nitrogen (harmful nitrogen, see above) (*Buchholz* et al., 1995; *Glattkowski* und *Thielecke*, 1995).

Under various environmental conditions (e.g. storage under elevated temperature) and due to wounding during harvesting, sucrose is enzymatically cleaved into invert sugar (glucose and fructose) in sugar beet, thereby reducing the sucrose yield and processing quality (*Hoffmann*, 2012). Methods for determining invert sugar were successfully established by KWS and others and later tested in automated beet laboratories (*Burba* et al., 1994). Immobilized enzyme assays using glucose oxidase and measuring the produced hydrogen peroxide via electrochemical reactions using an electrode or via spectrometry are commonly used methods today (*Rösner* et al., 1993). The serial analysis of the invert sugar content is nowadays part of the routine quality measurements undertaken in a number of sugar beet factories (*Vermeulen*, 2015). Since the levels of glucose and fructose do not occur in equimolar concentrations in the beet, glucose values have to be adjusted by different formulas in order to be used as a measure for invert sugar (*Schnepel* and *Hoffmann*, 2013; *Vermeulen*, 2015). ICUMSA has now an official method for invert sugar (ICUMSA, 2017 and 2019).

The most error-prone process in sugar beet quality analysis is the preparation of brei. Sugar content not only varies within one beet (*Burba* und *Haufe*, 1972a), but also between beet. To obtain reliable results for a given amount of sugar beet (e.g. a truckload) many samples from many beet should be taken. In light of this knowledge, sugar beet processing methods have been continuously modified, leading to the development of a specific beet saw in the 1960s. At the same time, various groups worked on automating all sugar beet laboratory processes. KWS developed an instrument to produce filtrated extracts out of frozen brei, the so-called "KWS Rundlauf" (*Reggelin*, 1965). At the same time, the Dutch company VENEMA successfully introduced a similar system to the sugar industry. In 1969, the first fully-automated sugar beet quality laboratory was built in a sugar factory in Tulln (Austria), with a capacity of 90 samples per hour (*Hartl*, 1970). Although other methods exist for analyzing sucrose, such as refractometry, gas- and liquid chromatography or photometric assays, polarimetry is the most commonly used method in the sugar industry (*McGrath* and *Fugate*, 2012). Polarimeters were continuously improved in the 20th century, leading to instruments, such as the Saccharomat® (Schmidt and Haensch (https://schmidt-haensch.com/products/laboratory-instruments/polarimeter/)), Sucromat® and BETALYZER® (*Kernchen*, 1976), SUCROLYZER® (Anton Paar GmbH (https://www.anton-paar.com)), which are now integral parts of sugar beet quality laboratories.

A method used for indirect sucrose determination is near-infrared spectroscopy (NIRS). NIRS is widely used for the estimation of agriculture quality parameters by simultaneously determining ingredients and by means of using nondestructive methods that does not involve sample handling. It is extremely rapid and is applied in labs, on harvesters and on mobile systems. Sugar industries worldwide, based on either sugarcane or beet, have an increasing interest in replacing cost- and time intensive laboratory procedures with fast and convenient NIRS methods. NIRS has been discussed for 30 years as a quality analysis tool in sugar beet processing (*Vaccari* at al., 1987; *Huijbregts*, 1996).

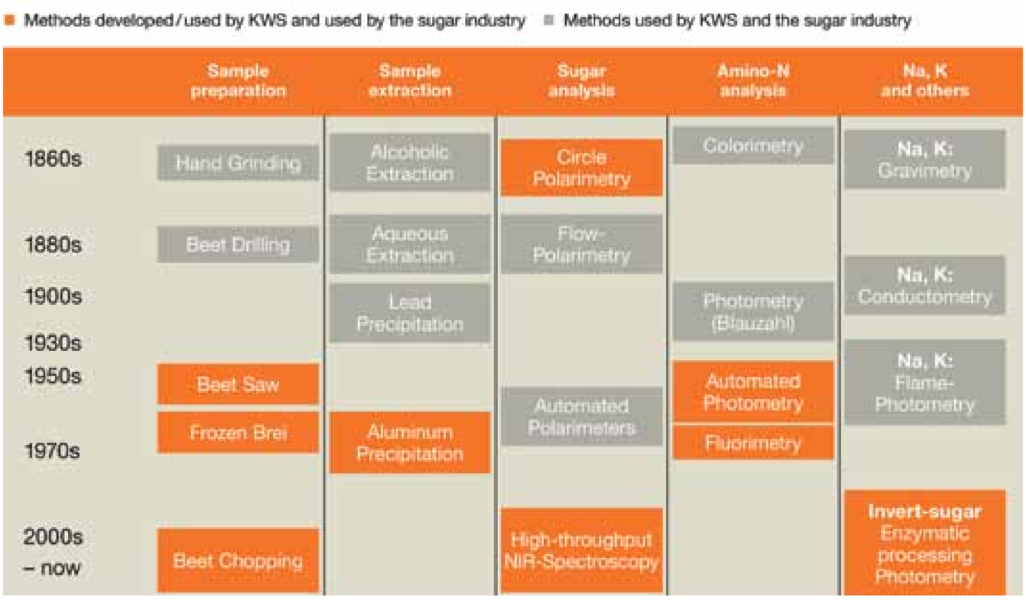

Fig. 1: Chronological overview of processing and analytical methods developed and used for sugar beet quality analysis
Na Sodium, K Potassium, NIR Near Infrared

on which methods are used for brei production and how the brei is processed. If brei processing is done by hand and not fully automated and standardized, the results might be prone to human error.

To reduce these bottlenecks, a new analyzer that allows for the measurement of the recoverable sugar content in sugar beet without the need for a laboratory was developed by KWS. Using this method, the beet are processed in a standardized, fully automated manner and analyzed via Polytec NIR-Spectrometer. These robust process spectrometers feature holographic grating technology, diode-array detectors, and no moving parts (Polytec-NIR-Spectrometers), allowing for extremely high throughput and precision while measuring in-flow. To optimize the results, different methods for sugar beet sample preparation were evaluated. An aqueous layer prevents penetration of NIR radiation in the sample. Therefore chopping beet has advantages over measuring brei or press juice.

This research led to the development of an innovative device that consists of a customized sugar beet chopper, which cuts 20–100 kg of sugar beet into small, uniform pieces (KWS Patent, 2009) (Fig. 2). These pieces are flattened and compressed

Today NIRS is used for quality analysis in e.g. sugarcane (*Madsen* et al., 2003) and for the analysis of sugar beet extracts, press juice and brei (*Huijbregts* et al., 2006), but also on whole sugar beet (*Panigrahi* and *Hofman*, 2003). NIR spectrometers can accurately measure sucrose in dark and colored samples and may eliminate the need for extract clarification (*McGrath* and *Fugate*, 2012). However, NIRS sugar measurements need to be calibrated carefully by using other standardized methods such as polarimetry. To obtain a representative dataset for calibration, sugar beet samples should be taken from different locations, years, disease pressures, varieties and agronomic practices (*McGrath* and *Fugate*, 2012). An overview of all commonly used methods described in the previous sections can be found in Figure 1.

4 Current developments

Most, if not all, current technologies for sugar beet quality analysis require a laboratory. This has many disadvantages, such as complex sample preparation, transport of samples to the laboratory, costly chemical waste management, the need for skilled manpower and relatively long waiting periods until results are available (see Fig. 2 for an overview). In addition, the quality of the sample preparation may vary significantly between different laboratories, depending

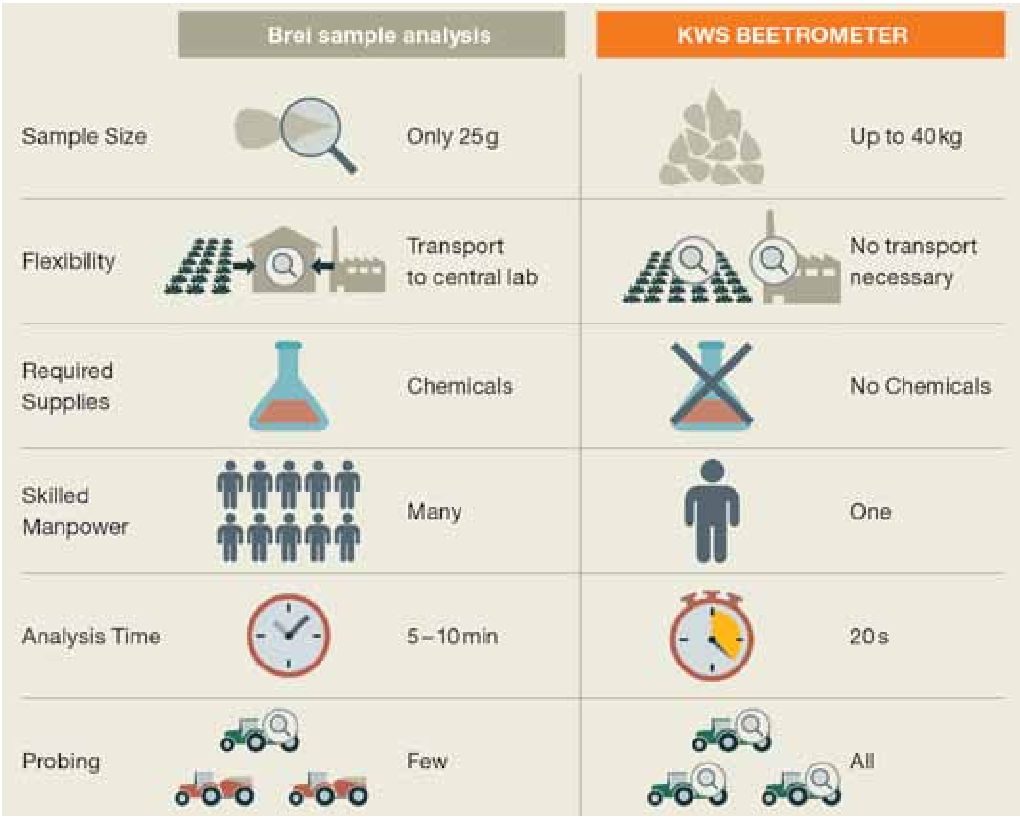

Fig. 2: Overview of requirements for conventional sugar beet brei analyses and benefits of the KWS BEETROMETER

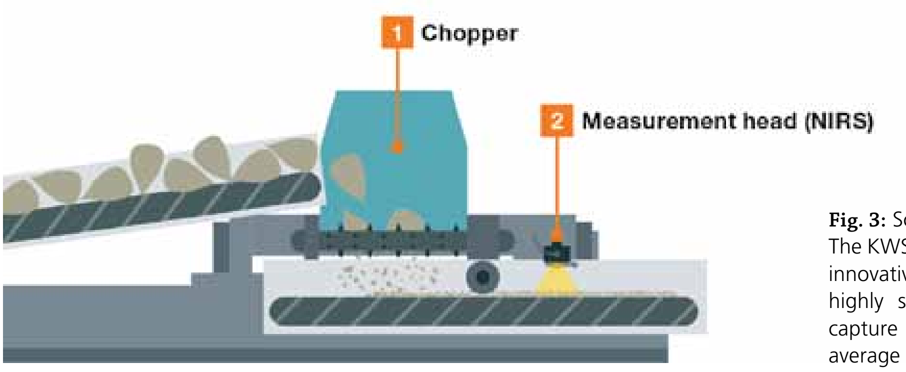

Fig. 3: Schematic representation of the KWS BEETROMETER. The KWS BEETROMETER[F400] is fully automated and combines innovative sample preparation technology (Chopper) with highly sensitive Near Infrared Spectrometer System. To capture the natural variation in beet sugar content, an average of 400 measurements are undertaken per sample.

by a roller to establish an even surface. A NIR spectrometer scans the surface of chopped beet as they move past, and 400 measurements are made per sample of 40 kg within 20 s (Polytec-PSS-Distance-scan-head) therefore capturing the full variation contained within a given sugar beet sample.
More than ten years of NIRS calibration with conventional methods in the quality laboratories of KWS and samples from all sugar beet growing areas worldwide have yielded a high level of accuracy when measuring the recoverable sucrose content via NIRS on freshly chopped beet (manuscript in preparation). This technique, developed originally for optimization of seed hybrids, is now integrated into the fully automated system called the KWS BEETROMETER[F400] ("F" stands for the possibility to use it as an integral part in the sugar beet processing factory and "400" stands for the average number of measurements per sample). It can be used on mobile devices in the field or installed wherever entire sugar beet need to be analyzed (stefan.meldau@kws.com)(Fig. 3). This system allows the recoverable sugar content and other sugar beet quality parameters to be automatically measured outside of the laboratory (e.g. in the field or at sugar beet storage facilities) and to increase the number of samples analysed from incoming loads of sugar beet at sugar factories.

5 Conclusions

Advances in sugar beet quality analysis have contributed to the success of the beet sugar industry for more than 200 years. Beside new developments in laboratory-based sugar beet analysis systems, high-throughput technologies such as the KWS BEETROMETER[F400] will offer new opportunities for breeders, sugar beet processing factories, harvesting technology providers and agronomists for analyzing the sugar content of beet. It is likely that the importance of methods for indirect measurements of sugar beet quality parameters, such as NIRS, will increase in the future. In addition, new methods will allow the analysis of further quality components in freshly chopped beet. Such methods will help to increase the profitability and competitiveness of the industry.

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Authors' addresses: Dr. *Stefan Meldau*, KWS SAAT SE, Business Development Sugar beet, Grimsehlstraße 31, 37574 Einbeck, Germany; e-mail: Stefan.Meldau@kws.com
Dr. *Manfred Burba*, Offenbachstraße 26, 37574 Einbeck, Germany; *Elke Hilscher*, KWS SAAT SE, Head of Chemistry, Grimsehlstraße 31, 37574 Einbeck, Germany