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Comparison of Dumas and Kjeldahl Method for Nitrogen Determination in Malt, Wort and Beer

Depending on the molar distribution, nitrogen has not only an important influence in yeast nutrition, but also on foam and haze stability of beer. Thus, it is important to have information on the nitrogen content of used grain, malt and produced wort. Over the years, various methods were described for the determination of nitrogen, with Kjeldahl and Dumas method being the most popular applications. However, according to literature, nitrogen contents of the methods are difficult to compare, because Kjeldahl method mainly measures organic nitrogen, whereas Dumas method determines total nitrogen content. Due to some advantages in terms of elimination of toxic reagents as well as shorter analysis time of Dumas methodology it would be a good alternative for application in the brewing industry. Comparison of the two nitrogen measurement methods was carried out with automated analyzers in barley malt (n = 408), wheat malt (n = 109) and wort or beer (n = 174). Besides a good repeatability of Dumas method (0.05 %) for barley malt, a significant correlation of r = 0.969 (P < 0.001) to Kjeldahl nitrogen could be determined. Comparable results were found for wheat malt (r = 0.932, P < 0.001) and wort (r = 0.981, P < 0.001). Variance analysis by means of t-test showed no significant differences in the repeated measurements between the two methods. Due to the good comparability as well as advantages in terms of occupational safety and faster analysis time, the Dumas method can be used for determining the nitrogen content in malt, wort and beer.

Descriptors: soluble nitrogen, protein, combustion method, Kjeldahl method

1 Introduction

Nitrogen has not only the highest percentage in air, but is also part of many organic molecules such as proteins or amino acids. These molecules are an important basis for various metabolic mechanisms and thus are of particular interest in beer production. Besides a small part from hops (protein content between 12 and 22 % dry matter) [17], main protein source in wort and beer are cereals like barley or wheat malt. According to *Narziss* and *Back* [16] protein content in barley ranges between 8.0–13.5 % (nitrogen 1.30–2.15 %), whereas content in malt drops by 0.1–0.5 % during malting procedure. While mashing an extraction and hydrolysis of these proteins results in different fractions (high, medium and low molecular proteins) in wort [15]. Coagulation and denaturation during boiling, removal of trub as well as flocculation and sedimentation in cold storage results in a further drop in soluble nitrogen content. Another portion of nitrogen is metabolized by the yeast to build up cellular protein, especially for enzyme and vitamin synthesis [2]. Thus, average nitrogen content is described with 700–800 mg/l in beer (12 °P original gravity) with an possible range between 180–1900 mg/l [13, 18]. Besides yeast nutrient, distribution of medium and high molecular weight nitrogen is decisive for colloidal and foam stability of beer. Since nitrogen has such an important role in brewing process, different methods for determination in malt, wort and beer were developed. These methods differ fundamentally in sample preparation and detection of nitrogen.

The oldest method was described by Dumas in 1831, whereas determination of nitrogen is performed by sample combustion in the presence of oxygen at 800–1000 °C, resulting in a conversion of nitrogen to nitrogen oxides. A catalytically reduction of these oxides to nitrogen gas allows a measurement using thermal conductivity detector (TCD) in combination with an electronic flow controller [4, 12]. Other combustion products like carbon dioxide, water or oxygen are removed by adsorption via several traps [4]. Thus, Dumas method allows a complete nitrogen recovery in solid and liquid samples, short analysis times with small needed sample amount as well as the elimination of toxic reagents [1]. However, since combustion method is no direct analysing technique, devices must be calibrated with standards of known concentration prior measurement.

A further method for determination of total nitrogen was proposed by *Kjeldahl* almost 50 years later [4]. In this case, digestion of the sample with concentrated sulfuric acid in presence of certain catalysts is carried out at high temperatures (> 380 °C). Organic nitrogen is reduced to ammonium sulfate, which are distilled as

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ammonia under the influence of sodium hydroxide into a template of boric acid solution of known molarity. Subsequently, the resulting ammonium borate can be measured directly or by titration [12, 14, 19]. According to literature, Kjeldahl method does not have good efficiency with substances like N-N- or N-O-compounds (nitrate or nucleic acids) as well as amino acids like tryptophan or lysine [4, 19]. Higher measurement accuracy of these compounds can be achieved through the use of copper (II) sulfate catalysts and the application of higher digestion temperatures (above 400 °C) [4].

Both, Kjeldahl as well as *Dumas* method have been tested by European Brewery Convention (EBC) and American Society of Brewing Chemists (ASBC) in collaborative trials in the 1990s [4, 6–8, 10, 11]. Furthermore there are ISO-standards for both measurement methods determining nitrogen content in cereals [20, 21]. Nevertheless, comparisons of methods did not show good comparability of the methods in the brewing sector. According to *Buckee* [4] both methods have a good reproducibility, but Dumas method gives slightly higher values in barley, malt and beer. However, only a sample size of 8 beer, malt and barley samples from a maximum of 25 laboratories were examined and the automated method was not performed by all participants. In this case, application of the automated Kjeldahl method compared to the manual method showed similar deviation (average nitrogen contents in barley N_{manual}=1.415 ± 0.028 % m/m (n = 17), $N_{automated}$ = 1.402 ± 0.035 % m/m (n = 10)), as the application of Dumas method (N_{Dumas} = 1.428 ± 0.030 % m/m (n = 20)) [4]. In addition, the average measurement error was less than 0.1 % m/m in each case. Later collaborative trials of the combustion method showed very good reproducibility (R_{95}: 0.116 % nitrogen of dry matter) and repeatability (r_{95}: 0.063 % nitrogen of dry matter), making this method an alternative to the Kjeldahl method [10, 11]. In comparison to these small sample sizes, large number of wheat samples in different harvest years were compared by *Seling* [19]. A good linearity between the methods was shown, however, a larger difference (0.4 % m/m) with higher protein content was found. Due to these differences, factors for the conversion of Dumas into Kjeldahl nitrogen were proposed. However, year-on-year, environmental and variety-related factors were shown to influence these conversion factors. Thus, conversion was discouraged. Comparable figures are not available for barley or beer. In addition, no precise methodology or use of measuring device was described and only single measurements were performed to compare nitrogen content of the wheat samples [19].

Based on these findings, aim of this study was a detailed comparison of the two measurement methods according to Kjeldahl and Dumas in brewing sector. In addition, not only wheat, but also barley malt and soluble nitrogen in wort and beer should be investigated to detect possible differences in nitrogen content. These studies are further intended to show whether conversion of nitrogen contents determined by Dumas in Kjeldahl are necessary.

2 Material and methods

2.1 Sample preparation

Barley and wheat malt samples (20 g) were grinded with DLFU-mill (Bühler Group, Braunschweig, Germany) with a grinding gap of 0.2 mm. Malt grist was homogenized and weighted on an analytical balance (Sartorius, Göttingen, Germany) with an accuracy of 0.1 mg. Liquid samples were pipetted into the sample vials and weighed.

2.2 Nitrogen determination according to Kjeldahl

Nitrogen measurement according to Kjeldahl were accomplished with Kjeltec™ 8400 (FOSS GmbH, Hamburg, Germany) with a previous sample digestion in the Foss Digestor Auto20 and Scrubber (FOSS GmbH, Hamburg, Germany).

Sample preparation for solid and liquid samples differed as follows: 1.0 g malt grist was weighted with 1 Kjeltab CT/5 (Foss Analytical A/S, Denmark) and 12 ml sulfuric acid (98 %) into the sample vessel. For wort and beer sample, 10 ml of sample with 1 Kjeltab CT/5, 8 ml sulfuric acid and 4 ml hydrogen peroxide were mixed and incubated for a reaction time of 30 min. This was followed by thermal digestion at 418 °C for 45 min. Further measurement was carried out with the automated system Kjeltec. Sample containers were transferred to the device and 30 ml distilled water as well as 30 ml sodium hydroxide solution was added, which was followed by the distillation into a template of 30 ml boric acid (20 g/l) and an indicator of bromocresol green and methyl red. Measurement of the color change via sulfuric acid (0.1 N) titration was carried out optically and the nitrogen content was calculated. Each sample was measured in duplicate. Protein content was calculated using a conversion factor of 6.25 for all samples, which is common in brewing industry [9, 25].

A blank test to detect N-compounds in the used reagents was performed once a week. In addition, a control of measuring accuracy by means of acetanilide was carried out.

2.3 Nitrogen determination according to Dumas

Combustion method was performed using rapid MAX N exceed analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). Since this technique is not an absolute determination method like Kjeldahl, content must be examined by means of calibration [9]. This calibration was performed with aspartic acid in a range between 95.06 µg and 76.05 mg nitrogen absolute. To check the device, a correction factor of 6 measurements aspartic acid was determined daily, which varied between 0.9 and 1.1.

The analyzer is composed of a combustion tube filled with copper oxide and corundum balls, a post-combustion tube filled with copper oxide, a platinum catalyst and a regainer, as well as a reducer tube which is prefabricated by the manufacturer. Quantitative

Table 1 Oxygen dosage time, flow and shutdown thresholds of the used analysis programs of rapid MAX N exceed analyzer

	correction factor	cereals	wort/beer
O_2-dosage time [s]	120	120	90
O_2-flow [ml/min]	500	175	150
O_2-shutdown threshold [%]	5	5	50
Maximum sample weight [mg]	600	500	2500

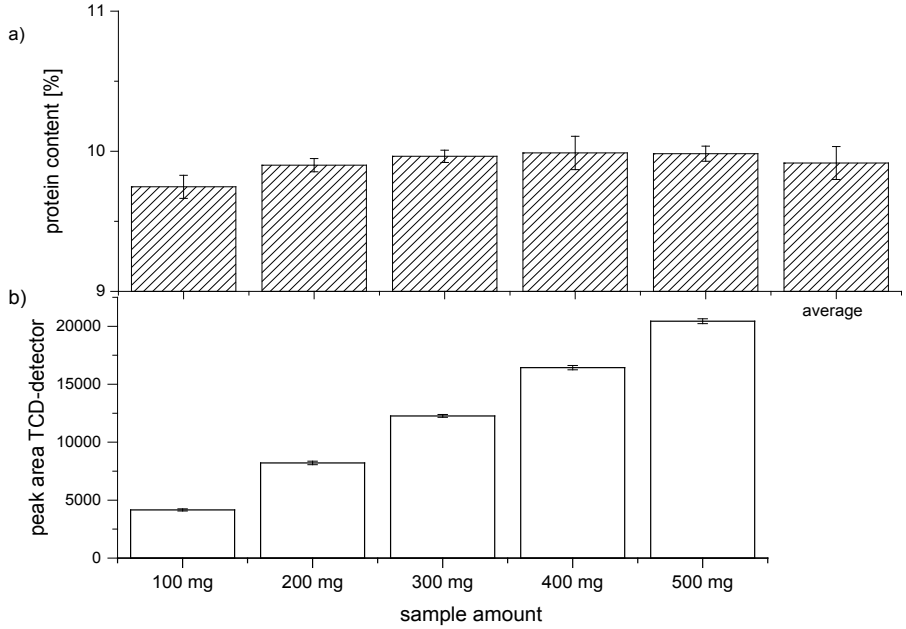

Fig. 1 Evaluation of the sample weight (n = 6) in a range between 100–500 mg on measurement result and standard deviation of TCD-detector peak area (a) and corresponding protein content (b) determined with rapid MAX N exceed (Elementar Analysensysteme GmbH, Langenselbold, Germany)

sample combustion is performed at approx. 900 °C. Nitrogen-free Helium (purity: 99.996 %, 3.8 bar) and oxygen (purity: 99.995 %, 2.5 bar) were used as the carrier and combustion gases. Oxidative combustion and separation of the further combustion gases is followed by a two-stage drying, TCD signal recording and result processing.

Analysis programs for investigation of cereals and beer are already specified by the manufacturer, which mostly differ due to the oxygen dosage rates and flow (see Table 1). In addition, maximum sample weights are suggested by the manufacturer. For nitrogen determination in barley and wheat malt, an optimal sample weight of 300 mg grinded cereals could be observed. Each sample was measured in triplicate. Sample weight with an accuracy of 0.1 mg was recorded and used for calculation of total nitrogen content. Similar procedures were used for liquid samples, whereas 2000 mg (accuracy 1 mg) beer and wort was used for analysis. Protein content was calculated using a conversion factor of 6.25 for all samples.

The absolute nitrogen content as well as the percentage protein content of the sample was calculated by the software rapidmaxn V1.1.0.

2.4 Statistics

Single measurement results of Dumas (n = 3) and Kjeldahl (n = 2) were compared using Pearson correlation. In addition, a t-test for analysis of variance (t-test with 2 samples and repeated measurement) and a Kruskal-Wallis test were performed for the comparison of the repeated measurements of both methods. Statistical analyses were carried out using OriginPro 2016G (OriginLab Cooperation, Northampton, USA).

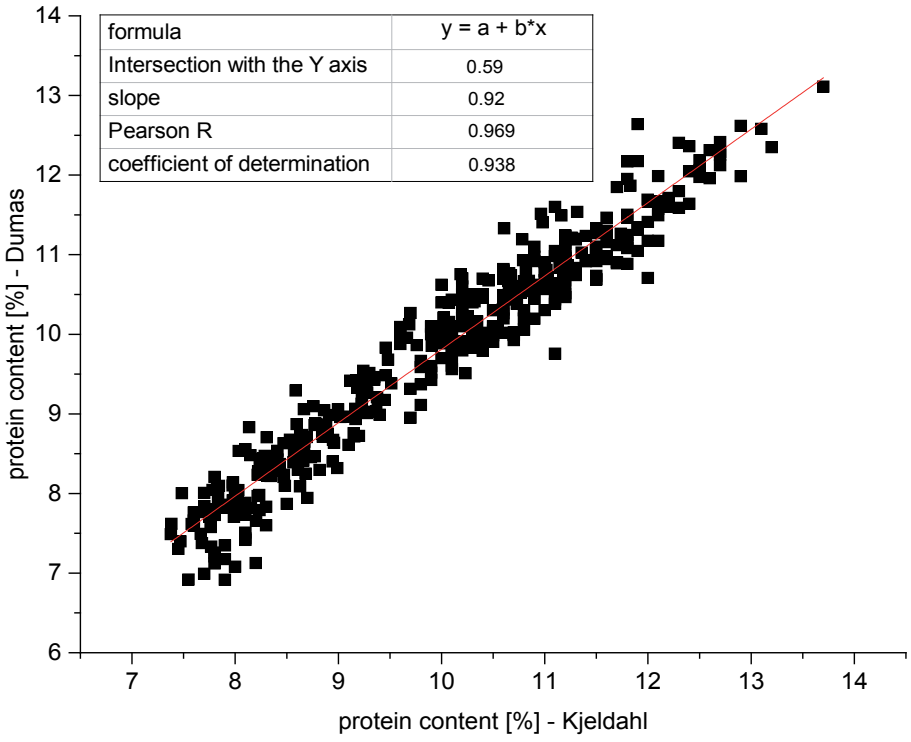

Fig. 2 Comparison of protein content (%, air-dry) of 408 barley malt samples analyzed according to Kjeldahl (Kjeltec 8400, Foss GmbH) (n = 2) and Dumas (Rapid MAX N exceed, Elementar Analysensysteme GmbH) (n = 3) method

3 Results and discussion

3.1 Method optimization

Before comparing the nitrogen measurement methods, an optimal sample weighing of solid and liquid samples for combustion method had to be determined. This was necessary because the instrument manufacturer merely describes a maximum sample amount (see Table 1) and existing methodology was based on an older generation of instruments with a lower accuracy [9]. Sample material was prepared according to EBC-Analytica 3.1 and 3.3.2 [23, 24]. First, a representative sample of barley malt was ensured using a sample divider (Retsch GmbH, Haan, Germany) and processed as described in chapter 2.1. After homogenization, grinded barley malt was weighed 6 times in a sample amount between 100 and 500 mg into sample containers and protein content was examined. Comparable trials were conducted with wort to determine soluble nitrogen in a sample amount between 0.5 and 3 ml.

Table 2 **Repeatability, maximum measurement error as well as coefficient of variation of protein contents in investigated barley (n=408) and wheat malts (n=109) as well as soluble nitrogen in wort and beer (n=174)**

		Dumas	Kjeldahl
Barley malt	Repeatability	0.05%	0.07%
	Maximum measurement error	0.47%	0.53%
	Coefficient of variation	0.005%	0.007%
Wheat malt	Repeatability	0.08%	0.09%
	Maximum measurement error	0.35 %	0.39%
	Coefficient of variation	0.011%	0.016%
Wort and beer	Repeatability	13.5 mg/l	13.3 mg/l
	Maximum measurement error	76.0 mg/l	118.7 mg/l
	Coefficient of variation	1.83%	2.49%

Reproducible measurement (±0.04%) results could be achieved starting at a sample weight of 200 mg for malt grist (see Figure 1a). For this reason, a minimum weight of 300 mg was used for the analysis of barley and wheat grist for all further experiments. Soluble nitrogen showed reproducible analysis starting at sample amounts of 1 ml, which is why a volume of 2 ml was chosen for further experiments (data not shown). The measured nitrogen contents should be lie within a medium calibration range, which was verified by the measured peak areas (see Figure 1b).

3.2 Data comparison

After evaluating an optimal sample weighting, the two methods were compared with barley and wheat malt as well as soluble nitrogen in wort and beer. The results observed a good comparability for barley malt. Figure 2 shows the comparison of the protein content (%, air-dry) determined by Dumas and Kjeldahl. A significant correlation with r = 0.969 (P<0.001, n = 408) for barley malt could be achieved. Linear regression resulted in a slope of 0.922. Protein content of barley malt samples ranged between 7 and 13%.

Repeatability (n = 408×3) of Dumas method was 0.05% with a maximum measurement error of 0.47% in protein content (see Table 2). Comparable results were obtained for the Kjeldahl method for barley malt (see Table 2).

In addition to barley malt, protein content of wheat malt was investigated, shown in figure 3. Comparison of Dumas and Kjeldahl resulted in a significant correlation of r = 0.932 (P<0.001, n = 109). Measurement range of the protein content was examined between 9.0 and 13.5% (air-dry). Linear regression could be calculated with y = 1.022x–0.0526. Compared to barley malt, a slightly larger scattering of the measured data could be observed. Reasons for the larger scattering can be found mainly in the grist texture (wheat malt grist was coarser than barley malt) and a resulting inferior homogeneity. In addition, there are basically differences in the amino acid and protein composition of wheat and barley and the associated distribution of e.g. N-N- and N-O- components [3].

Repeatability of Dumas method could be determined with 0.08% in protein content of wheat malt and 0.09% using Kjeldahl method (see Table 2). Besides protein content of solid samples, soluble nitrogen of wort and beer was investigated. Nitrogen content ranged between 600 and 1600 mg/l, wherein most samples had a nitrogen content between 600–1100 mg/l (Figure 4, see page 22). Comparison of Kjeldahl and Dumas nitrogen resulted in a significant correlation of r = 0.981 (P<0.001). Combustion of liquid samples had a repeatability of 13.5 mg/l with a maximum measurement error of 76.0 mg/l.

Kjeldahl method had a repeatability of 13.3 mg/l in wort and beer (see Table 2). Slope of linear regression of the method comparison was determined with 0.899.

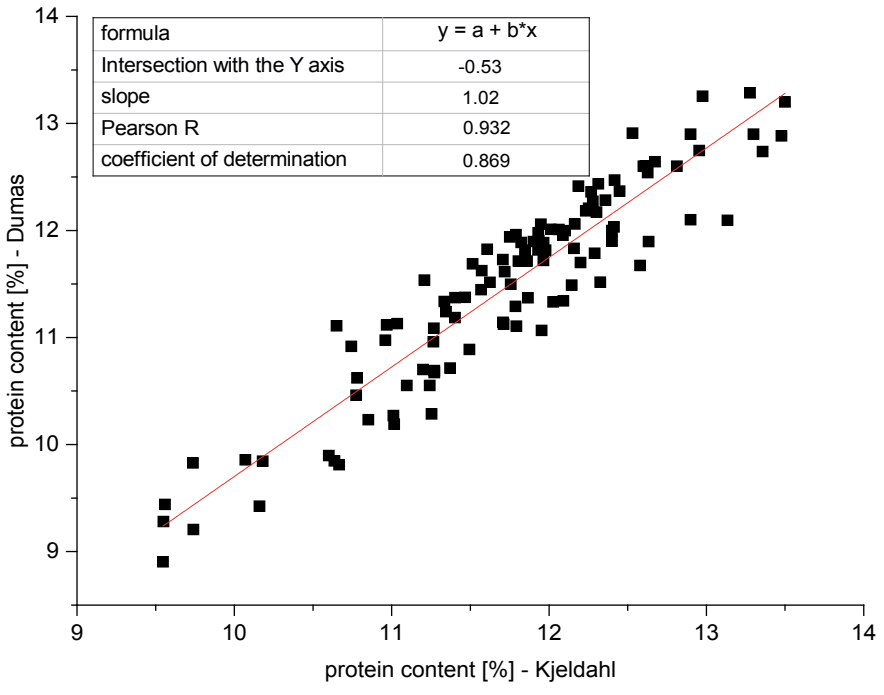

Fig. 3 **Comparison of protein content (%, air-dry) of 109 wheat malt samples analyzed according to Kjeldahl (n = 2) and Dumas (n = 3) method**

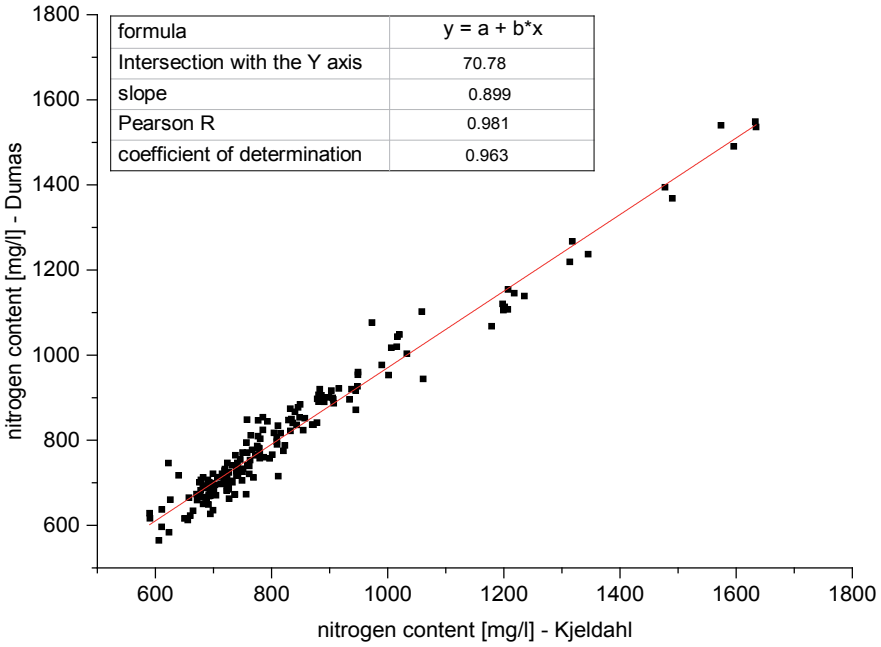

Fig. 4 Comparison of soluble nitrogen content in wort and beer samples (n=174) analyzed according to Dumas (n=3) and Kjeldahl (n=2) method

Nitrogen content in malt and wort showed a good comparability over the entire investigated measuring range. Compared to literature, higher nitrogen contents determined with combustion method could not be confirmed. Rather, a scatter around an average value is evident. This is apparent above all in the malt samples due to the straight line slope near angle bisector. For this reason, in addition to the correlation, an analysis of variance of the repeated measurements was carried out to investigate deviations in the total nitrogen content between the performed methods. The results showed that both t-test and Kruskal-Wallis-test did not detect significant differences between the repeated measurements of Dumas and Kjeldahl method. Thus, possible deviations in nitrogen contents can be explained by measurement inaccuracies as well as weighing errors or inhomogeneity in the sample material.

Observed data show that the use of nitrogen analyzers determine comparable concentrations in malt and wort. Thus, findings from the literature on higher values of Dumas nitrogen cannot be confirmed. However, these findings were based on smaller sample quantities or single measurements [4, 19, 22]. In addition, in the case of wheat, higher nitrogen ranges were investigated, which are not relevant for the brewing industry [19]. *Foster* [6] described a good comparability in barley malt with no significant difference at a 99 % confidence level of the analyzing methods in malt samples. However, comparable figures could not be found either for wheat malt or soluble nitrogen in wort and beer. The presented results show that the further development of the measuring devices as well as the applied calibration and verification procedures of Dumas method resulted in a good comparability with Kjeldahl nitrogen. Thus, Dumas method is an acceptable alternative to the Kjeldahl method for analyzing malt, wort and beer. Conversion of the results determined with combustion method are not necessary. Advantages of the combustion analyzer are shorter analysis times as well as an avoidance of the use of toxic reagents like sulfuric acid and sodium hydroxide [1]. Structural advantages due to no need for laboratory fume hoods or acid scrubbers and a less required space make the Dumas method easy to implement in all laboratory environments [6]. These advantages increase safety in everyday laboratory practice and also has a positive impact on the environment [5].

4 Outlook

Since a widely use of Kjeldahl method in brewing industry and described deviations of the measurement results in literature, Dumas method has not been established in German brewing industry. However, these deviations can no longer be observed with the use of new measuring instruments. In addition, Dumas method has a multitude of advantages. Above all, the potential of the small sample amount and the short analysis time which allows the application of Dumas devices in an automated laboratory environment. This offers new opportunities for analysis, especially in the field of research.

Nevertheless, Kjeldahl method has advantages in the processing of salty or fatty samples due to easy handling. This is important, for example, for the detection of high molecular weight nitrogen by means of magnesium sulfate precipitation. Thus, there are still wide fields of application for both methods in the brewing industry. Depending on the required result, both methods can be used in brewing area with a high accuracy and repeatability.

5 Literature

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