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PRECISION AND UNBIASEDNESS OF AN OVEN METHOD AND KARL-FISCHER-TITRATION TO DETERMINE THE SEED MOISTURE CONTENT

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A b s t r a c t. The different methods of determining the moisture content result in values with different precision and unbiasedness. The significance of grinding pea seeds during sample preparation on the accuracy of the moisture content was quantified. For working samples with particle sizes of 0-1, 0-2, 0-3, 0-5 and 0-7.5 mm a mean moisture content of 13.56, 13.47, 13.43, 13.39, and 12.25 %, respectively and a variance of 0.013, 0.009, 0.008, 0.007 and 0.092 $\%^2$, respectively, were determined with an air-oven method. For working samples with particle sizes of 0-1 mm a mean moisture content of 13.00 % and a variance of 0.011 $\%^2$ were determined with a Karl-Fischer-Titration. The significance of over and under estimations due to water loss or gain during sample preparation, chemical reactions during the analysis and residual water in the matrix, the influence of the homogeneity of the moisture content within a sample and the particle size distribution on the precision of a moisture determination will be discussed.

K e y w o r d s: seed moisture content, oven method, Karl-Fischer-Titration, pea seeds

INTRODUCTION

For the study of many biological and physical properties of seeds, the knowledge of the moisture content is essential. Today a wide range of methods to determine the moisture content, using the different chemical and physical properties are known [7,11,20]. The appropriate method however will be designated by the required accuracy, the time and funds available and laboratory equipment.

For the official seed testing an air-oven method is used as a reference method [14]. Detailed instructions of the procedure during the analysis and of the necessary equipment constitute these international rules. However, even determinations which strictly adhere to these rules give different values as each step during the whole analysis entails inaccuracies. These variance components are the cause for values with different precision and unbiasedness. The more strict the rules are formulated and applied, the better will be the precision and therefore the repeatability of the values found with smaller variances between repetitions. Principally an oven method should achieve a reduction in oxidation and decomposition and the loss of nonaqueous volatiles, whilst ensuring the separation of as much water as possible during the drying period. As this method must also be suitable for routine use, larger tolerances concerning the parameters of the rules must be allowed and therefore larger tolerances in accuracy.

Grinding seeds is recognised as being an important inaccuracy factor, especially for seeds for which pre-drying is obligatory. The following experiments shall demonstrate

the significance of the fineness of the ground material on the accuracy. Additionally, with a Karl-Fischer-Titration (KFT) a method was applied to research the unbiasedness of oven method values.

MATERIAL AND METHODS

Moisture determinations were conducted with pea seeds (*Pisum sativum* L.) being stored in closed glass bottles at 5 °C over a period of at least 4 weeks. The seed moisture content varied within the seed lot with a variance of $s^2=0.12\ \%^2$ [2].

The moisture determination with a standard air-oven was conducted following the 'International Rules for Seed Testing' at a temperature of 130 °C for 1 h and calculated on a wet mass basis [14]. Working samples of 4-5 g were ground in a cross beater mill model Culatti from Janke & Kunkel. Different ranges of particle sizes of the ground material could be obtained by using different mesh bottoms with round holes of 1, 2, 3, and 5 mm in diameter, respectively. If no mesh bottom was used, it was possible that a few seeds were only chipped and could retain their original sizes of up to 7.5 mm. Additionally, working samples of whole seeds were dried in the air-oven for 16 h. During grinding the open containers were put directly underneath the outlet of the grinding chamber. The mill was opened and sucked out after each run. The mass was measured with an analytical balance with a resolution of 0.1 mg.

The moisture determination with the Karl-Fischer-Titration [19,23] was carried out with an automatic titrator model Titroprozessor 682 from Metrohm with standard equipment (motor driven burette, double platinum electrode, magnetic stirrer). The titration vessel was equipped with a thermostatically controlled jacket. The water was extracted with 30 ml methanol per working sample within the titration vessel at 50 °C. For the volumetric titration the single-component reagent Hydranal-Composite 5 from Riedel-de Haen was used. For the electrometric endpoint detection a double platinum electrode was used with a constant current of 50 mA. The potentiometric titration was stopped if the polarization was smaller than 250 mV for 30 s. The water equivalent of the reagent was determined by titrating known amounts of sodium tatrate-2-hydrate. The reagent consumption was measured with a resolution of 2 ml. During titration a deposit can cover electrodes, which could influence the current flow. Therefore the electrodes were cleaned after 5 titrations. For the fine grinding a vibration disc mill from Siebtechnik with a closed grinding chamber with a net volume of 322 ml, equipped with 2 discs of steel, was used. The mass of the weigh-in quantity, which were in increments of about 200 mg out of 50 g grist transferred through a weighing scoop into the titration vessel, was determined with a resolution of 0.1 mg.

The particle size was determined by a screen analysis using a laboratory sieving machine model Vibro from Retsch with test sieves of wire gauze and rubber cubes as screening aids. The measured grinding period was the time during which mechanical comminution took place. To determine the temperature a thermometer model Metratherm 1200d from BBC was stuck on the grist of additional samples.

From each experiment 10 repetitions were analysed. The comparison of mean values was conducted with the Behrens-Fisher-test and the comparison of variation was conducted with the variance ratio test at a significance level of $\alpha=0.05$ [10].

RESULTS

The mean moisture content and variance of pea seeds depending on the determination method and the range of particle sizes of the analysed material is shown in Table 1. Regarding the mechanical comminution, for the working samples with particle sizes of 0-1, 0-2, 0-3, 0-5 and 0-7.5 mm a time of about 7, 2, 1-2, 1-2 and 1 s, respectively were needed and parts of the grist were warmed up by about 4, 4, 4, 3 and 1 °C respectively. Fine grinded peas had approx. 83 % particles smaller

Table 1. Accuracy of the moisture content of pea seeds depending on the determination method and the range of particle sizes of the analysed material

Fineness	Air-oven						KFT
	Coarse grinding					Whole seeds	Fine grind.
Range of particle sizes (mm)	0 - 1	0 - 2	0 - 3	0 - 5	0 - 7.5	5 - 7.5	0 - 1
Mean (%)	13.53a	13.47ab	13.41b	13.38b	12.32e	12.64d	13.00c
Variance	0.013b	0.012b	0.008b	0.007b	0.089a	0.028ab	0.011b

a-e Means and variances respectively with different letters are significantly different.

than 0.16 mm and 17 % particles between 0.16 and 1 mm.

DISCUSSION

With the increasing fineness of the material to be dried a higher moisture content was measured. With increasing fineness an increasing probability exists for water to escape from the material during the comminution due to its larger specific surface area increased through put time and increased temperatures in the mill. The parameters in the 'International Rules for Seed Testing' for grinding allow a very wide range of particle sizes. The stipulation that at least 50 % of the ground material shall pass through a sieve with a mesh of 4.00 mm would even allow a wider range of particle sizes than tested in this experiment. By definition all mean values of the ground material being dried in the oven are true values. Without further analysis however, it is not possible to say which one is the best value when referring to the reduction of oxidation, decomposition and the loss of nonaqueous volatiles whilst removing as much water as possible during the drying period.

All oven methods are based upon the determination of the mass of a working sample before and after the drying process. As the measured differences do not differentiate between escaping volatiles and water with most oven methods the term moisture content is used instead of water content. During the drying process chemical reactions take place resulting in over and under estimations. The loss of nonaqueous volatiles and the formation of water by maillard-reactions falsely indicate a higher water content [8,16]. Residual water in the matrix at the end of the drying process and chemical reactions as oxidation or water consumption through dextrine formation falsely indicate a lower water content [4,5,20]. These chemical reactions depend mainly on the quality of the sample, the drying temperature and time. The extent of the final over or under estimation is very difficult to quantify.

With the KFT a method can be used which is based on a water specific reaction. For most seeds non-water specific reactions during the titration can be neglected [19,23], but water loss or gain during sample preparation and residual water in the matrix after the titration has stopped can also occur during any KFT [3]. This must be considered if a KFT value is to be used for comparison with an oven method value [1,6,9,16,21,22].

Even with modern titration techniques like instrumentation delay or calculation of the drift strict standardizations are necessary. However, with the KFT, a method can be used to quantify some chemical reactions being responsible for over or under estimations of oven methods. Therefore the use of a tube furnace for water separation and the titration of water in an inert gas or air could be suitable, as this type of water separation is basically the same as in an oven. The significance of the condition of the sample

(species, variety, moisture content or particle size), the drying temperature and time for chemical reactions in the form of escaping nonaqueous volatiles or oxidation, could be studied by the use of the tube furnace technique. The separation of water by heat may require less experience in terms of handling during sample preparation, but the problems of residual water still must be considered.

Leroy [15] quantified the necessary energy C by the following relation:

$$C = RT_c \log (P_o/P)$$

where R marks a constant, P the vapour pressure of the medium at the temperature T_c and P_o the vapour pressure of the medium of saturated vapour at T_c. As the term P_o/P tends to infinity when P tends towards zero, infinite energy would be necessary to separate all water from the matrix. Under practical considerations definite quantities of water are measured as the resolution of the instruments is limited [17]. The 'International Association of Cereal Chemists' [12] takes into consideration this problem of endpoint detection and has introduced further restrictions. Hereafter all water is considered separated if the difference between two values does not exceed a defined value if the second measurement is carried out 48 h later. This underlines the fact that the moisture content is always defined by a method and that standardized methods are necessary.

An important quality aspect of a method is also the precision of the measured values. For the determination of the precision, repetitions with identical working samples are desirable. The used pea seeds were relatively homogeneous, as the moisture content between the single seeds varied with a variance of only 0.12 %^{2}. For a working sample of 4-5 g 20 seeds were used, which meant that the seeds themselves caused a variance of about 0.006 %^{2}. The experiments demonstrate that grinding is an important variance component. The smallest variance with 0.007 %^{2} was measured if the particle sizes ranged from 0 to 5 mm and the highest variance with 0.089 %^{2} was measured when the particle sizes ranged from 0 to 7.5 mm. With the use of whole seeds an improved variance could not be obtained. Matthews [18] found a similar precision for wheat seeds with average variances of 0.008 %^{2} for ground seeds and 0.017 %^{2} for whole seeds. Paynter and Hurburgh [21] measured for ground maize seeds a variance of 0.011 %^{2} and 0.052 %^{2} for whole seeds. The use of whole seeds would have the advantage that the variance components as grinding and pre-drying do not occur. On the other hand the precision of the measurements depends more on the physical properties of the seeds which can cause large variance especially with relatively big pea seeds. Detailed experiments of the particle size distributions and the temperature distributions of the samples can possibly contribute to the explanation of this effect.

It is desirable to obtain a grist with a standardized particle size distribution through grinding. More restrictive parameters concerning the particle size distribution are formulated in the regulations of the 'International Association of Cereal Chemists' or the 'International Organization for Standardization' [12,13].

Using 2 repetitions for a moisture determination of a sample at a significance level of α=0.05, tolerances in a laboratory of 0.24 to 0.82 % can be expected. As grinding can be conducted by different ISTA-laboratories within a wide range, relatively large tolerances between laboratories of about 2 % must be expected. It is necessary to research the precision and unbiasedness of moisture determinations practically measured. An enquete which concerns itself with the variance between laboratory results and the moisture distribution within a seed lot could give valuable data on the accuracy of this difficult measurement.

CONCLUSION

A KFT may be used to research over and under estimations of moisture determinations

with an air-oven. For precise determinations strict standardizations and continual enquetes are necessary.

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