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Foodstuffs - Sample comminution for mycotoxins analysis - Comparison between dry milling and slurry mixing

Produits alimentaires - Préparation d'échantillons gros volume pour l'analyse des mycotoxines - Comparaison entre broyage à sec et broyage par voie humide

Lebensmittel - Probenvorbereitung für die Mycotoxinanalytik - Vergleich zwischen Trockenvermahlung und Aufschlämmung

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Foreword

This Technical Report (CEN/TR 15298:2006) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal method”, the secretariat of which is held by DIN.

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Introduction

Since 1999-01-01, EC directives for aflatoxins entered into force, which consisted of sampling plans resulting in sample weights of up to 30 kg. This raised questions on how these relatively big samples could fulfil the requirement to “finely grind and mix thoroughly each laboratory sample using a process that has been demonstrated to achieve complete homogenisation” [1]. Since the analytical sample is taken out of this big sample, the critical step is to take a representative increment out of it. As such this topic has been subject of several studies in the past. Dickens and Satterwhite [2] developed a mill that could handle up to 25 kg peanut samples. They presented results of tests on 5 kg samples from which they withdrew 50 g sub-samples, but gave no data on larger samples. Velasco and Morris [3] considered use of a water slurry to obtain finer particles and a more uniform particle distribution. Another advantage of slurry preparation is the avoidance of clogging of samples that have high oil content. They presented experiments on different matrices with sample weights up to 4,5 kg, whereas they mentioned that slurry preparation is limited only by the capacity of the equipment. Whitaker et al. [4] considered a compromise. They prepared a slurry from a sample, which was first comminuted by another milling process. Due to the regulations of the USDA they limited themselves to an amount of only 1 100 g. Nevertheless this restriction in their method was developed into the alternative best foods method used for aflatoxin in peanuts [5]. Dorner and Cole [6] started all over again from the beginning: the 218 kg sample of raw, shelled peanuts for analysis in official USDA approved laboratories. They compared variability by grinding with four different mills, but only with sub-sample sizes up to 4 kg. So the question how the result would be on 21,8 kg samples remained unanswered. Their statistical data, especially CV values, on the 2 kg and 4 kg sub-samples were less favourable than the ones that can be achieved by applying the slurry method. Scholten and Spanjer [7] published data on slurry preparation for samples up to 10 kg, whereas the laboratory of Wiertz, Eggert and Jörissen had similar experiences, even when applying samples up to 30 kg. Data of the latter are compiled in this report. Worldwide however, sub-sampling mills are in favour because they are easy to apply and fast in comminuting samples into analytical portions. Calori-Domingues et al. [8] demonstrated this with a poster presentation at the Xth International IUPAC symposium on mycotoxins and phycotoxins in May 2000. They tested variability for aflatoxin analysis in peanuts associated with sample preparation by dry milling with a RAS mill. Unfortunately however they only investigated samples up to 5 kg.

So the labs of the Inspectorate for Health Protection, a delivery unit of the Dutch Food and non-food Authority, and of Wiertz, Eggert and Jörissen, a member of the Eurofins Scientific group, decided to perform new experiments with following goals: 1. what CV values are achieved when milling 10 kg samples, and 2. are correct aflatoxin values measured while doing so? The choice of matrices has been discussed at a CEN/TC 275/WG 5 (Comité Européen de Normalisation, Technical Committee 275, Working Group 5, Biotoxins) meeting, considering existing and upcoming legislation for different mycotoxins and food types. Combining both items lead to the conclusion that a lot of matrices, existing as dried, whole or ground raw material are to be considered. Also differences in sample weight, i.e. between nuts and spices, exist. Suggestions for representative commodities were:

— cereals, since for this staple food directives exist on as well as aflatoxins, as ochratoxin A and as DON;

— raisins, because these are included in directives for aflatoxins and ochratoxin A;

— paprika powder as an example of a ground commodity.

In practice however it turned out that the availability of naturally contaminated lots that could be used for these experiments was the limiting factor. The presented results show what exactly has been examined. After these experiments the detailed work of Schatzki and Toyofuku [9], who measured particle size distributions on pistachio slurries, became available. This lead to a joint presentation at the 2nd World Mycotoxin Forum, February 2003, in The Netherlands [10]. This report is a combined outline of both investigations.

1 Scope

A comparison was made between dry milling and slurry mixing as comminution step preceding mycotoxins analysis. Such in respect to EC legislation that consists of sample schemes up to 30 kg. Cacao, green coffee, almonds and pistachio samples of 10 kg were milled by a RAS mill and all three sub-samples were completely analysed for aflatoxin B_1 or Ochratoxin A. The differences in analytical results are explained by measurements of particle size distributions of both milling types. The obtained data are compared with literature data on coefficients of variation (CV) for various milling procedures. For dry milling CV values were generally not below 20 % for aflatoxin B_1 levels up to 38 µg/kg in peanuts, whereas slurry mixing could achieve CV values below 5 % at aflatoxin B_1 levels down to 4 µg/kg in pistachios. Measurements also showed possible difference in mycotoxin content of a sample between both milling types. This could lead to false positive or negative results when rejecting or accepting a lot, as this is based on the sample result. It was concluded that slurries contain smaller particles than dry milled samples and thus generate the lowest possible CV values which in turn leads to better sample homogenisation.

2 Test methods

2.1 Apparatus

2.1.1 Slurry mixer, Slurry mixer - Silverson type EX mixer ® [1)];

2.1.2 RAS mill, Romer Analytical Sampling mill ® [1)]

Other laboratory equipment and slurry preparation procedures as described before (see [7] and [9]). The RAS mill was applied according to the manual (Release 2, January 1998) of the supplier. Before the dry milling process the pistachio samples were frozen overnight at minus 20 °C.

2.2 Reagents and materials

Aflatoxin measurements were performed as described in EN 14123. Ochratoxin measurements were carried out in cacao and in green coffee beans as described in EN 14132, including quality control. The only difference is that fluorescence detection for ochratoxin A is carried out as published by Zimmerli and Dick [11].

2.3 Procedure

For each commodity, experiments were carried out by the following procedure:

1. sampling according to the EC directive, resulting in 10 kg sample;
2. milling the 10 kg sample by a Romer mill with a split ratio of 10 %;
3. taking a dry sample out of the 10 % part as usual for Romer mill users (sub-sample A);
4. slurry mixing of the remaining part of the 10 % part of the sample (sub-sample B);
5. slurry preparation of the 90 % part by Silverson mixing (sub-sample C);
6. analysing the three sub-samples A, B and C by HPLC methods.

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[1] Silverson type EX mixer is the trade name of a product supplied by Silverson Machines Ltd., Waterside, Chesham, Bucks, England. Romer Analytical Sampling (RAS) mill is the trade name of a product supplied by Coring-System Diagnostic GmbH, Robert-Bunsen-Straβe 4, D-64579 Gernsheim, Germany. This information is given for the convenience of the users of this Technical Report and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.