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Homogenization of breakfast cereals using cryogenic grinding

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Abstract

Ready-to-eat breakfast cereals are important source of micronutrients despite the losses of these elements during processing. From an analytical point of view, these materials are complex heterogeneous samples containing as many as 13 constituents. It is critical that the sample be representative and grinding of the sample is essential before taking a sub-sample for subsequent steps in the analytical procedure. In this work the use of cryogenic grinding was studied as a fast and effective procedure for sample comminution and homogenization. This procedure is efficient and the powders obtained were acid-digested using a microwave-assisted procedure. Iron, manganese, and zinc were determined by flame atomic absorption spectrometry.

Keywords: Sample preparation; Cryogenic grinding; Breakfast cereals

1. Introduction

The sample homogeneity is a problem in trace element analysis of heterogeneous complex materials and may compromise precision and accuracy of the results. Modern instrumental analysis uses samples with low masses. For attaining a representative sample with low masses it is essential to comminute samples to decrease heterogeneity. Samples can be comminuted using mechanical devices. Usually, the mechanical procedures for sample homogenization can contribute to metal contamination from the grinding apparatus and volatile constituents can be lost owing to the excessive heat generation (Markert, 1995). One procedure for particle size reduction and homogenization is cryogenic grinding, also called the "brittle fracture technique". Iyengar and Kasperek (1977) studied the distribution behavior of elements at different concentration levels in biological matrices using this technique. The precisions obtained for the determination of Fe, Mn, Rb, Se, Zn, in the $\mu g\ g^{-1}$ range, and Ag and Co, in the $ng\ g^{-1}$ range, varied for homogenized and non-homogenized bovine liver samples, with higher values obtained for non-homogenized samples. For samples homogenized by cryogenic grinding the precisions for these elements were similar to those obtained for macroconstituents, such as K, P, Cl and Na present in $mg\ g^{-1}$ range. Later, De Boer and Maessen (1980) applied this technique for grinding human placenta samples. These authors compared the efficiency of particle reduction using two different cooling media: dry ice-acetone (−78°C) and liquid nitrogen (−196°C). They demonstrated that grinding at lower temperature led to smaller particle sizes. Zeisler, Langland, and Harrison (1983) demonstrated the efficiency of a cryogenic grinder for homogenization of liver, adipose, and muscle tissues.

Other important aspect is the amount of sample that can be treated in the cryogenic grinding equipment. Although not critical for quantitative chemical analysis taking into account the small masses needed, the mass is crucial for preparation of reference materials with strict guarantees of homogeneity as discussed by Rossbach, Ostapczuk, and Emons (1998). This issue was addressed by developing cryogenic grinding equipment able to comminute up to 3 kg of sample per hour (Kramer & Pauwels, 1990). A new high-capacity device for cryogenic grinding was recently developed by Singh and Goswami (1999), and its performance was evaluated for

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grinding spices without losses of volatile oils and flavouring components.

According to May and Kaiser (1984) the main features of the cryogenic impact grinding technique are protection of the hot-labile components, short grinding time per sample, incorporation of lyophilization to facilitate handling and storage, and easy, rapid cleanup of the equipment. These authors emphasized that the main problems were contamination of samples with Cr, Fe, Mo, and Ni and the limited size of the container.

The present work proposes a rapid cryogenic grinding procedure to reduce particle sizes of breakfast cereal samples. Booth, Reilly, and Farmakalidis (1996) commented that the processing of whole food cereal grains to the final product results in losses of all minerals.

2. Experimental

2.1. Apparatus

Samples were prepared using a Model 6750 Freezer Mill impact grinder with Model 6751 grinding vials, (Spex Certiprep., USA), and with a self-contained liquid nitrogen bath (4–5 l). The cylindrical grinding vial assembly consisted of two ferromagnetic 440C stainless steel end plugs, a polycarbonate center section, and a 440C stainless steel impactor. The performance of this device was compared to a conventional knife grinding Willey type, model TE-048 (Marconi, Brazil). A microwave furnace equipped with PFA closed vessels (Milestone, ETHOS 1600, Germany) was used for sample decomposition. Iron, Mn and Zn were determined in the diluted digested solutions by flame atomic absorption spectrometry (FAAS, SpectrAA 640, Varian, Australia). All measurements were made with an air–C_2H_2 flame, and the instrumental conditions are showed in Table 1. Particle size measurements were made using a scanning electron microscopy, Model LEO StereoScan 440, (Cambridge, UK).

2.2. Reagents and samples

All solutions were prepared from analytical reagent grade using Milli-Q® water with a conductivity lower than 18 mΩ cm^{-1} (Millipore, USA). Concentrated solutions of analytical grade HNO_3 and H_2O_2 (Mallinckrodt, Mexico) were used for sample decomposition. Reference solutions containing Fe, Mn, and Zn were prepared from stock solutions containing 1000 mg l^{-1} of each metal (Merck, Germany).

Four different breakfast cereal samples were employed for evaluating the performance of the grinding procedure. According to the sample labels the major constituents were: oat flakes, wheat fibers, corn flakes, cashew nut, peanut, wheat grain, Brazilian nut, and brown sugar (sample 1); oat flakes, wheat fibers, corn flakes, cashewnut, peanut, wheat grain, Brazilian nut, brown sugar, and raisin (sample 2); roasted oat, corn flakes, oat flakes, sugar, raisin, rice flakes, dried apple, wheat bran, malt powder, honey, iodized salt, grated coconut, and wheat grain (sample 3); and oat, sugar, raisin, and ground cinnamon (sample 4).

2.3. Procedure

A 1-g sample mass was inserted in the polycarbonate tube equipped with a stainless steel magnetic bar. The polycarbonate tube was closed using stainless steel end plugs. An alternating magnetic field was applied and the magnetic bar impacts each extremity of this tube according to the frequency of the field applied. This movement causes the grinding of the sample.

The tube was placed in the mill container filled with liquid nitrogen. A two-step grinding program was applied. Samples were initially cooled for 2 min before the grinding for 2 min. The samples were ground using a frequency of 20 Hz.

After grinding, 300 mg of each sample was transferred to the PFA microwave vessels. Three ml of conc. HNO_3 plus 0.5 ml of conc. H_2O_2 solutions were added to each vessel. The microwave heating program was implemented in four successive steps with a total cycle time of 11 and 12 min for breakfast cereals and corn samples, respectively (Table 2). The digests were quantitatively transferred to 25.00 ml volumetric flasks and the volumes were made up with water. Four replicates were prepared for each breakfast cereal sample.

The effect of manipulation and the stainless steel magnetic bar on contamination was evaluated using three different grinding times (60, 120 and 180 s) and with 2 min for initial cooling. This experiment was made

Table 1
Instrumental parameters adopted for determination Fe, Mn and Zn by FAAS

Parameter	Fe	Mn	Zn
Wavelength (nm)	248.3	279.5	213.9
Slit width (nm)	0.2	0.2	0.7
Lamp Current (mA)	30	5	15

Table 2
Microwave-assisted acid-digestion program for breakfast cereals and corn samples

Step	Time (min)	Power (W)
1	1.5	250
2	1.5	0
3	3.0–4.0[a]	250
4	5.0	400–500[a]

[a] Corn sample.

with a corn sample owing to the hardness of its raw seeds.

The effect of particle size on sample homogeneity was investigated by determining Fe, Mn, and Zn by FAAS in a cereal sample with and without grinding.

For comparison two samples of breakfast cereals were also ground in a knife mill using 10 g of each sample. This mill was furnished with a 25-mesh sieve.

The distribution of particle sizes obtained using both mills was determined by scanning electron microscopy. The measurements were carried out suspending the pulverized sample in ethanol and using this suspension onto a brass sample cavity holder. The sample surface was recovered with a gold film for improving electron conduction and resolution.

3. Results and discussion

The cryogenic homogenization procedure converts the solid frozen tissue into a powder. Frequently the total time involved is less than 5 min and the efficiency of the process is dependent on the matrix characteristics of the sample. Samples containing high contents of water, which are hardly comminuted by mechanical devices at room temperature without a previous drying step, can be easily pulverized by cryogenic grinding. Despite this possibility, it is recommended to dry or to lyophilize samples before grinding to obtain the results based on dry mass. In another study in progress, the cryogenic grinding showed unsatisfactory action only for samples containing high contents of oils or fats, as observed for Brazilian nuts that contain 68.2% of fat (Holland et al., 1991). May and Kaiser (1984) also observed that the lipid content of fish samples caused powder clumping after cryogenic grinding.

3.1. Evaluation of sample contamination

Considering the contact between samples and metallic parts of the cryogenic grinder, an experiment was performed to evaluate contamination. Owing to the difficulty of simulating a blank sample with hardness similar to the samples, an experiment was designed based on the effect of increasing the grinding time on the contents of Fe and Zn. These elements were chosen because they were determined, and contamination affects critically the determination of both at trace levels. The results obtained are shown in Table 3. A Q-test for rejection of results showed that all results are statistically acceptable at a 95% confidence level indicating that there is no increase in the contents of Fe and Zn caused by contact with metallic parts. Groups 1 and 2 refer to experiments made with two different sets of grinding cylinders, end plugs, and magnetic bars.

Table 3
Determination of Fe and Zn in corn seeds sample using different times of grinding

Time (s)	Fe (μg g^{-1})		Zn (μg g^{-1})	
	Group 1	Group 2	Group 1	Group 2
60	27.5 ± 0.1	27.5 ± 0.1	19 ± 2	22 ± 2
120	30 ± 3	31 ± 2	20.8 ± 0.2	21 ± 2
180	29 ± 2	27.4 ± 0.1	20 ± 1	23.4 ± 0.2

3.2. Effect of sample homogeneity

Breakfast cereals are complex samples containing many constituents characterized by different chemical compositions, hardness, and sizes. The contents of Fe, Mn and Zn were evaluated for the sample 1 after and before cryogenic grinding. Without grinding the relative standard deviations were high owing to sample heterogeneity (Fig. 1). This is clear indication of the expected effect of sample heterogeneity. For complex samples, it is only possible to obtain representativity for a small mass after reducing the particle sizes drastically. For a 300-mg cereal sample, the presence of one raisin can represent up to 25% of the total mass and exerts a pronounced effect on the elemental composition.

3.3. Cryogenic and knife-mill grinding: particle sizes

Two different types of breakfast cereals, with (sample 4) and without (sample 1) raisins, were evaluated considering particle sizes obtained using the two tested grinding procedures: cryogenic and knife-mill. The latter mill was chosen owing to its wide use in the preparation of organic samples.

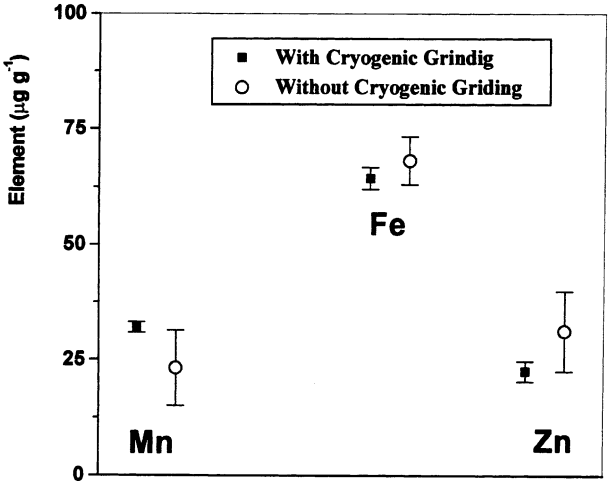

Fig. 1. Determination of Fe, Mn, and Zn in breakfast cereal: effect of homogeneity on the mean and standard deviation values ($n = 4$) obtained with and without cryogenic grinding. The standard deviations are represented by the vertical bars.

The grinding procedures using the knife-mill and the cryogenic devices used 10 and 1 g of sample, respectively. Ten successive grindings of each sample were performed with the cryogenic mill, and the powders were mixed to guarantee suitable representativity and to obtain enough sample mass for all experiments carried out.

Breakfast cereal samples containing raisins were difficult to grind using the knife mill, because water and fibers formed a paste that stick at the knives and affected their performance, requiring the interruption of the process and the manual cleaning of the knives before restarting. According to Holland et al. (1991) raisins contain 13.2% and 6.1% water and fibers, respectively. This was completely overcome in the cryogenic grinding procedure owing to the preliminary cooling step that avoided any paste formation for these samples.

Fig. 2 obtained by scanning electron microscopy (2000× magnification), shows the particle sizes attained with each procedure for sample 1. Cryogenic (Fig. 2(a)) and knife mill (Fig. 2(b)) procedures generated similar particle sizes. Particles for both samples, after applying both grinding procedures, had a diameter of around 30 μm. However, it cannot be assured that the powders obtained by each procedure suitably represented the composition of the original sample owing to the effects above cited. Some results obtained for Fe, Mn, and Zn in samples comminuted using the knife mill device indicated negative errors and high standard deviations compared to those obtained using the cryogenic grinding procedure. Particle shapes were similar using both mills and most particles had an irregular spherical shape.

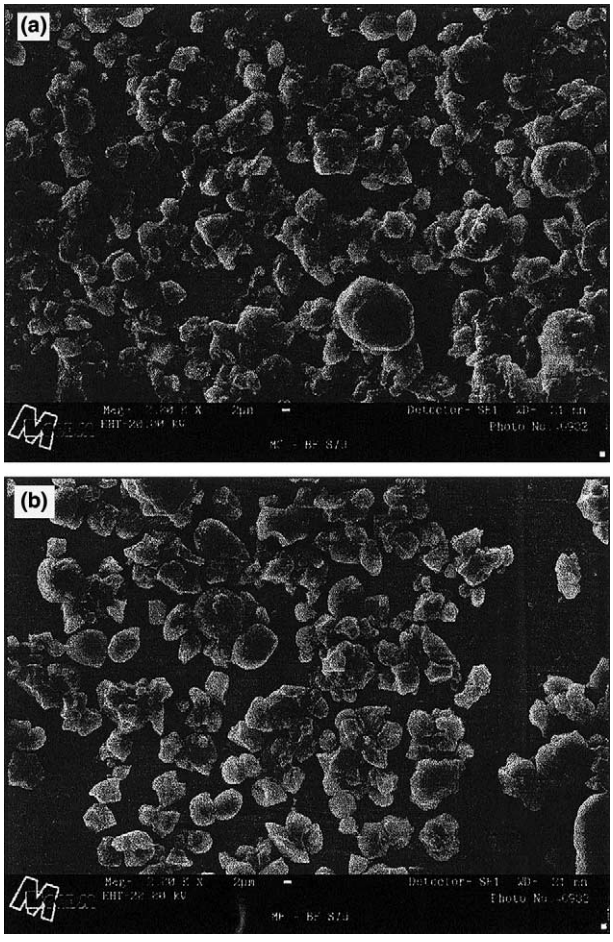

Fig. 2. Scanning electron microscopy of particles generated for breakfast cereal sample 1 after grinding. (a) Cryogenic mill and (b) knife mill.

Table 4
Determination of Fe, Mn and Zn in breakfast cereals combining cryogenic grinding and microwave-assisted acid digestion

Sample	Fe (μg g^{-1})	Mn (μg g^{-1})	Zn (μg g^{-1})
1	64 ± 2	32 ± 1	22 ± 2
2	66 ± 1	16.4 ± 0.3	17 ± 1
3	60 ± 3	8.6 ± 0.6	75 ± 3
4	46 ± 1	21.5 ± 0.7	9.5 ± 0.3

3.4. Determination of Fe, Mn, and Zn combining cryogenic grinding and microwave-assisted decomposition

Four samples were cryogenically ground and the powders were acid-digested using a closed-vessel microwave-assisted procedure. The results obtained are shown in Table 4. The accuracy of the procedure cannot be checked using standard reference materials because there are no standards with composition similar to breakfast cereal samples and additionally these standards are normally sold as powders ready to be dried and digested, and not as raw materials to be ground. Thus the suitability of the cryogenic grinding procedure cannot be proved by this approach. The standard deviations were lower than those obtained with the knife mill indicating a better homogeneity without critical effects caused by sample constituents or sample manipulation.

Cryogenic grinding is an effective and fast procedure for proper particle size reduction. Contamination effects need to be better evaluated for other food sample materials. Working with harder samples and more sensitive analytical techniques, such as graphite furnace AAS, the contamination problems may become critical. May and Kaiser (1984) suggested covering the ferromagnetic impact bar with a polymer, such as PTFE, resistant to liquid nitrogen temperatures, but it is not sure that this material can withstand such a low temperature.

4. Conclusion

Cryogenic grinding is a very effective procedure for fast sample particle size reduction. The occurrence of contamination can be a critical issue, but this is also true

for other milling devices. Both the particle sizes and the homogeneity obtained from the cryogenic grinding procedure are useful characteristics that can also be exploited to preparation of slurries for direct introduction by auto-sampler or pneumatic nebulization. Investigations based on this approach are in progress.

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