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Reproducible Sample Preparation for Reliable Food Analysis

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Abstract

A reliable and accurate analysis of food samples can only be guaranteed by reproducible sample preparation. This chapter describes the process of turning a laboratory sample into a representative part sample with homogeneous analytical fineness by choosing the most suitable mill. Important aspects of size reduction and homogenization are explained, a variety of application examples is given, and specific applications such as cryogenic grinding are discussed in detail.

Keywords: sampling and sample division, sample preparation, homogenization, pulverization, milling, cryogenic grinding, minimizing standard deviation, particle size

1. Introduction

Food occurs in a great variety of consistencies and is often inhomogeneous. Food testing laboratories require representative samples to produce meaningful and reproducible analysis results. Therefore, food samples have to be homogenized and pulverized to the required analytical fineness, ideally with as little time and effort as possible. Furthermore, reliable analytical results can only be obtained if the entire sample preparation process is carried out reproducibly [1]. This means that the prepared part sample, from which usually only a few grams or milligrams are required for analysis, needs to represent the laboratory sample as well as the original sample from which the laboratory sample was extracted. An inhomogeneous sample does not represent the original material because some components may be overrepresented or missing altogether. Consequently, a homogeneous sample is the basis for reliable and representative analytical results. A good example to understand the importance of sample homogeneity is fat analysis of pizza. Only a few milligrams of pizza are required for

analysis. Random sampling might result in a piece of mushroom or salami or cheese which would falsify the total fat content in the subsequent analysis (**Figure 1**). However, if the pizza is first reduced to coarse particles of <5 mm and then pulverized to fine particles of <0.5 mm, a homogeneous, representative analysis sample is obtained.

Only a homogenized analytical sample fully represents the initial sample and ensures reliable and reproducible results—independent from which spot the part sample is taken. The standard deviation of any subsequent analysis can be minimized drastically by particle size reduction and homogenization of the analytical sample. In the pizza example, the fat content was measured (**Figure 2**). The fat content varies in the pizza samples with particle sizes around 5 mm, whereas it is much more consistent in the homogenized samples. The standard deviation (SD) is reduced from 0.21 to 0.03% (relative SD from 2.10 to 0.35%).

Figure 1. From left to right: a whole pizza; sample after grinding to coarse particles of <5 mm; fully homogenized sample with particle sizes of <0.5 mm.
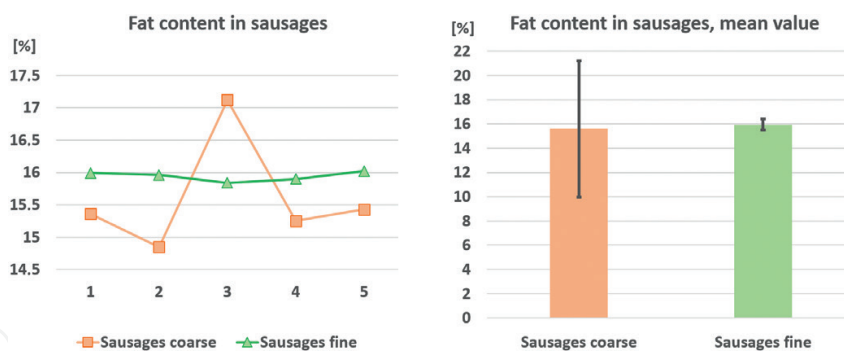

Figure 2. Left: fat content varies in coarse pizza samples but is stable in the pulverized samples; right: the mean values of each batch of five samples, the relative standard deviation of the fat content is reduced from 2.10 to 0.35% by homogenization.

2. How to select a suitable laboratory mill and accessories

When searching for a suitable mill and grinding tools, one should keep in mind that the sample properties to be determined (such as moisture or heavy metal content) must not be altered in any way during the process. To find the best suited mill for a specific application, the following aspects should be considered in advance:

- Feed size and required final fineness (Section 2.1).

- Characteristics of the sample and size reduction principles (Section 2.2).

- Sample volume and sample throughput (Section 2.3).

- Grinding tools and subsequent analysis (Section 2.4).

- Drying or embrittlement of the sample (Section 2.5).

2.1. Feed size and required final fineness

The feed size means the original particle size of the sample. For choosing a suitable mill, it makes a great difference whether large samples, like a whole fish, or small particles, such as crop grains, are to be homogenized. Whereas samples with small particle sizes can be fed directly into most grinders and mills, large-sized samples do not fit into every mill. Therefore, manual size reduction, for example, by cutting, or a preliminary grinding step in another mill may be required. The mills that accept larger initial particle sizes are mostly not suitable for producing very fine particles which are small enough for subsequent analysis. A frequent requirement is to "grind the sample to fine powder," but the term "powder" is not precise [2]. Washing powder, coffee powder, or baking powder have very different particle size distributions. Another typical request is to have the sample ground "as fine as possible." This involves a high input of energy and time which in turn increases costs. A much more effective approach is to grind the sample as fine as necessary. The required analytical fineness of the sample material depends on the analytical method or further processing and can vary greatly. Most methods require a fineness in the size range from 20 µm to 2 mm. As product properties (e.g., extraction, filtration, or absorption capacity) are often influenced by the particle size, size reduction on a laboratory scale is also essential for the development of new products or production processes.

2.2. Characteristics of the sample and size reduction principles

Depending on the sample properties, different size reduction principles are suitable to obtain the required fineness [3]. As mentioned before, large particles cannot always be ground to analytical fineness in one step. In some cases, it is possible to carry out preliminary and fine grinding in the same mill with different settings; in other cases, two mills are required. Another essential aspect relates to the sample properties: to produce a size reduction effect, the comminution principle of the mill should match the breaking behavior of the sample. Therefore, when selecting a suitable instrument, a thorough evaluation of the sample material is essential. Properties such as density, hardness, consistency, residual moisture, or fat content must be considered. Other characteristics, which may influence the success of the grinding process, are temperature stability or tendency of the sample to agglomerate.

Laboratory mills work with different size reduction principles. The type of mill used always depends on the breaking properties of the sample material. The subsequent pages show the most common mechanisms for the size reduction of solids. Usually, various size reduction principles are combined in one mill, such as impact and friction in planetary ball mills or shearing and impact in rotor mills. Hard and brittle samples are pulverized best by pressure,

impact, and friction. These size reduction principles, however, have only limited effect on fibrous, soft, elastic samples. Pulverizing a freeze-dried fish, for example, is not possible by using pressure or impact; cutting and shearing are suited much better.

2.2.1. Size reduction of hard and brittle materials

Hard and brittle materials can be crushed with pressure, impact effects and/or friction (**Figure 3**). Pressure means a force which is applied between two solid surfaces that either represent the grinding tool surfaces directly or may be the surfaces of adjacent particles. Pressure is exerted by the grinding tools (jaw crushers, toggle crushers). Impact means a force at a solid surface. This could either be that of a grinding tool, or be represented by other particles. Strain by impact is mainly caused by one-sided and opposing particle acceleration (mixer mills, planetary mills, impact mills, jet impact mills, and drum mills). Friction means a force between two solid surfaces, caused by the vertical pressure of one surface and the simultaneous movement of the other surface (mortar grinders, disc mills, hand mortars, and rod mills).

2.2.2. Size reduction of soft, elastic, and fibrous materials

Shearing and cutting mechanisms are best suited to pulverize soft, elastic, and fibrous materials (**Figure 4**). Shearing means a force between two or more solid surfaces moving in opposite
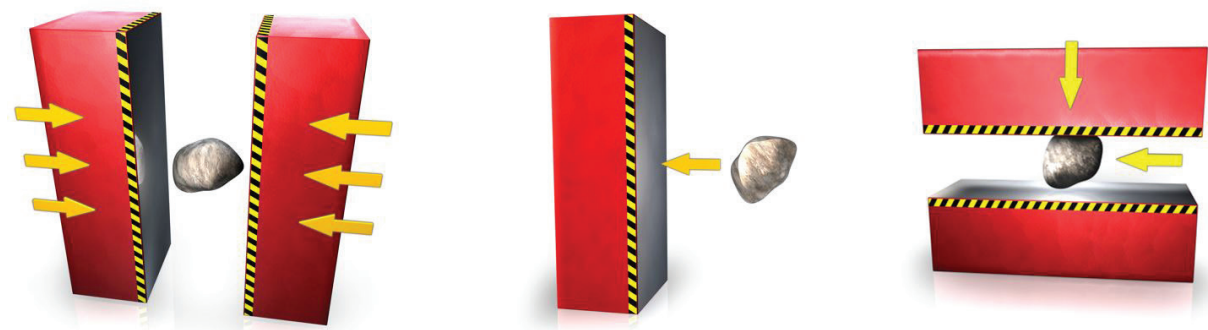

Figure 3. Size reduction principles for hard and brittle materials: pressure, impact, and friction.
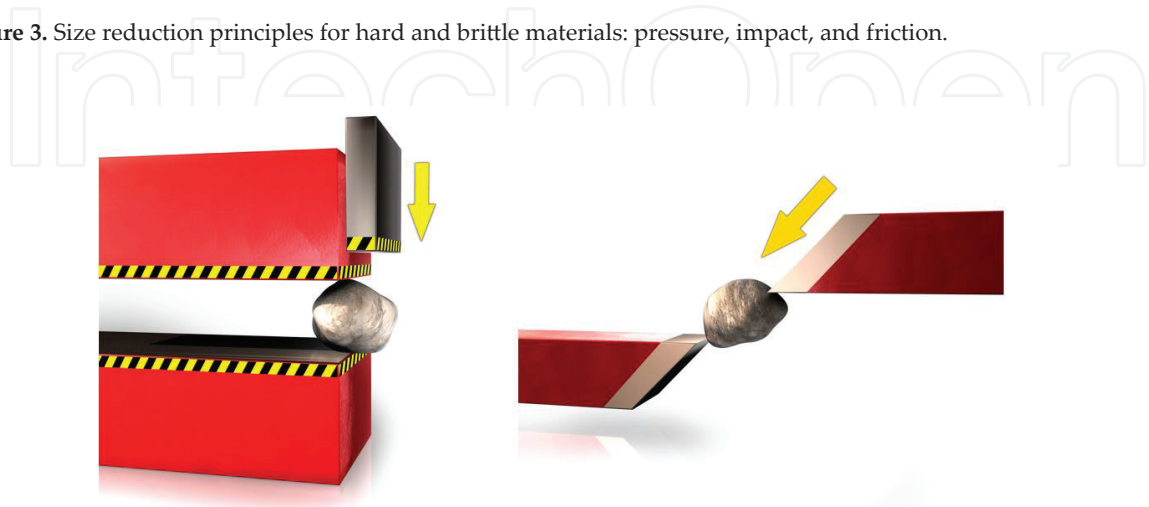

Figure 4. Size reduction principles for soft, elastic, fibrous materials: shearing and cutting.

directions (rotor beater mills, cross-beater mills, and ultra centrifugal mills). Cutting means a force between two or more sharpened surfaces (shredders, cutting mills, and knife mills).

2.3. Sample volume and sample throughput

Some mills accept larger sample quantities than others. Open systems with an inlet and outlet, like rotor mills, may be fed with larger amounts of bulk material. However, if the mill has a closed grinding chamber, for example, the grinding jar of a ball mill, the sample amount which can be processed in one batch is limited. Grinding kilogram quantities of wheat in a rotor mill is carried out much quicker than grinding the same amount in a mixer mill with a maximum jar volume of 50 mL (sample amount of 20 mL). The sample throughput may also influence the choice of a mill. It is based on the time required to assemble all parts of the mill, to grind the sample, and to clean the mill between two different samples. If only a few samples are analyzed each day, increased effort for a singular sample preparation process may be tolerable but not if hundreds of samples need to be prepared on a daily basis.

2.4. Grinding tools and subsequent analysis

For most mills and crushers, a variety of accessories and grinding tools are available. The selection of suitable accessories ensures effective grinding processes and reliable results. Two aspects should be considered: Which accessories are most effective and how the subsequent analysis might be influenced by the material of the grinding tools. Grinding tools are available in different materials, depending on the type of mill. The most common are the following:

• Metal (steel, cast iron, titanium).

• Ceramics (tungsten carbide, zirconium oxide, sintered aluminum oxide, hard porcelain, glass).

• Natural stone (agate).

• Plastics (PTFE, PC, PP, PE).

Grinding tools made of steel are available for all mills. When choosing a suitable grinding set, several factors must be considered, such as the hardness of the sample material and its breaking properties. The material of the grinding set should be harder than the sample to avoid excessive wear. In the case of food samples, this is true for most of the grinding materials used. Another important feature, which is mostly relevant for ball mills, is the energy input generated by the different materials. Grinding balls of tungsten carbide, for example, generate a much higher energy input, and thereby a better size reduction effect, due to the higher density of the material, than balls of the same size of other materials. On the other hand, too much crushing efficiency leads to caking of the sample material on the jar walls, especially in ball mills. This applies to soft, fatty, and sticky materials, characteristics which are typical for food samples. Therefore, the energy input must be considered carefully to avoid these effects.

Mechanical size reduction always leads to a certain degree of abrasion which may influence the subsequent analysis. Consequently, traces of materials like steel or zirconium oxide may

be found in the sample. Anyhow, the amount is usually below detection limit for most analyses and can therefore be neglected. Moreover, some analyses, for example, determination of the fat content, are not affected by the iron and chromium traces resulting from steel abrasion. If, however, the heavy metal content is the object of investigation, the abrasion coming from steel equipment may lead to falsified results. In this case, using tools made of a "neutral" material like zirconium oxide or tungsten carbide is more advisable. The degree of abrasion also depends on the sample properties and the size reduction principle of the mill.

A special case is sample preparation under cryogenic conditions [4]: grinding with dry ice or liquid nitrogen should only be carried out with tools that are completely made of stainless steel. Plastic tools are not suitable as plastic embrittles at very low temperatures and may be damaged.

Regardless of the grinding tool material, the correct choice of accessories can have substantial influence on the grinding efficiency. For example, when grinding salad in a knife mill, the efficiency is greatly increased by using a gravity lid instead of a standard lid. Salad loses volume while being ground, and the gravity lid pushes the sample down against the knives for continuous homogenization.

2.5. Drying or embrittlement of the sample

2.5.1. Drying

It is only possible to grind moist or even wet sample materials without undesired side effects and sample loss with knife mills. When ground in rotor mills, moist materials tend to block the sieves which can lead to a blockage of the machine. As a consequence, material is lost and much time has to be spent on cleaning the mill. Therefore, it is advisable to dry the material before further processing. When choosing the drying method and temperature, care must be taken that the properties of the sample to be determined are not altered in any way. That is especially important with regard to temperature-sensitive or volatile components. Usually, these types of sample can only be air-dried at room temperature. Fluid bed dryers are suitable for gentle and quick drying of many products with an average drying time of 5–20 min. Further methods include vacuum and freeze drying as well as drying in ovens.

2.5.2. Embrittlement with liquid nitrogen or dry ice

Cooling the sample material often improves its breaking behavior. Some soft, tough, sticky, and fatty food materials have to be cooled before they can be subjected to preliminary or fine-size reduction. Chocolate or raisins, for example, can be pulverized easily by cryogenic grinding, whereas at room temperature, it is only possible to produce a paste with a low homogeneity. One way is to embrittle the sample in liquid nitrogen (LN_2) before grinding. At a temperature of −196°C, even soft jelly bears become so hard and brittle that they are pulverized without problems. Another possibility is to mix the sample with dry ice (solid CO_2). If the sample contains volatile substances which must be preserved for analysis, cryogenic grinding is also the method of choice. However, materials which must not become moist should not directly be treated with cooling agents, because the humidity of the air is condensing on the

cold sample. Cooling agents should never be used in closed grinding tools as evaporation causes overpressure in the jar. More details of cryogenic grinding will be discussed at the end of this chapter.

3. Overview of mills commonly used for food sample preparation

Before specific application examples are discussed, we give an overview of the different mill types which are used most commonly for food sample preparation:

- Rotor mills (ultra centrifugal mills, cyclone mills, rotor beater mills).

- Knife mills.

- Ball mills (mixer mills, cryo mills).

- Cutting mills.

Obviously, more than one mill type may be suitable for grinding a particular sample, for example, wheat. As mentioned before, the choice of the most suitable mill for a certain sample depends on the sample volume, the required final fineness, the throughput, the material properties, and the subsequent analysis. The knowledge of the basics and working principles of different mill types helps to make the optimum choice for a specific application.

3.1. Rotor mills

Typical applications include seeds, corn, maize, wheat, dried algae, salt, sugar, dried fish, peas, nuts, almonds, coconut, coffee, tea, roots, gelatin, dried leaves, rice, spices, herbs, soya meal, and so on.

All types of rotor mills share the same grinding principle. The sample enters the mill through a hopper, hits on a rotor, which is either placed horizontally or vertically in the mill, and is smashed with impact onto the rotor teeth. In the second step, the sample passes a sieve with a specific aperture size. Here, mostly shearing effects are applied, with exception of the cyclone mill where friction prevails. Finally, the sample is collected in a bottle, cassette, or receptacle. In the following, three different types of rotor mills are discussed: ultra centrifugal mill, cyclone mill, and rotor beater mill.

3.1.1. Ultra centrifugal mills

Ultra centrifugal mills are used for the rapid fine-size reduction of soft, medium-hard, brittle, and fibrous materials. Size reduction is effected through impact and shearing forces between ring sieve and horizontal rotor. The maximum feed size is 10 mm. Especially with maximum speed, but depending on the material, a final fineness of 40 μm (d_{90}) and below may be achieved. Among the rotor mills, this is the highest achievable fineness. The grind size is determined by the aperture size of the exchangeable ring sieves (usually ranging from 0.08 to 10 mm). The revolution speed of ultra centrifugal mills ranges from 6000 to

18,000 min^{-1} or even more. The cassette principle guarantees 100% sample recovery and easy cleaning. It is recommendable to use a vibratory feeder for automatic and uniform feeding of large amounts of free-flowing materials. If large quantities or temperature-sensitive materials are processed, the use of a cyclone, for example, with a 3- or 5-L collector, is recommended. The frictional heat that is generated during the grinding process is partly discharged through the cyclone, so it helps to cool the sample. The use of distance sieves instead of standard ring sieves also helps to reduce frictional heat due to the greater gap between sieve plate and rotor. Accessories for ultra centrifugal mills usually include ring sieves and rotors of titanium for heavy-metal-free size reduction. If hard and abrasive materials are to be ground, a rotor with abrasion-resistant coating is required. For processing small amounts of sample, a mini-cassette with matching 316-L stainless steel rotor and various ring sieves is suitable.

Tips and techniques:

- When grinding an unknown sample, it is advisable to start with a sieve with a medium aperture size. The aperture size may be reduced if the sample does not block the sieve. This applies to all rotor mills.

- Grinding in ultra centrifugal mills is very effective: as a rule of thumb, 80% of the pulverized sample is smaller than half the aperture size of the sieve.

- If the sound of the machines changes significantly and/or if dusty material suddenly comes out of the hopper, the grinding chamber must be checked for overload or blocked sieves.

- When grinding temperature-sensitive materials, a cyclone helps to reduce the temperature. Distance sieves have the same effect.

- If the sample is fatty, the use of a distance sieve is advisable, as the shearing effect is reduced, and consequently less fat is "squeezed" from the particles which might block the apertures.

- Large particles should be pre-crushed using a sieve with medium to large aperture size. Fine grinding in a second step using a finer sieve is mostly quicker than trying to force large particles directly through the small apertures.

- If sample material remains in the grinding chamber although a cyclone is used, removing the sieve and letting the mill run for a few seconds clears the chamber. Repeat this step from time to time during milling of large quantities.

3.1.2. Cyclone mills

Cyclone mills are specially designed for the processing of foods and feedstuff for subsequent near-infrared spectroscopy (NIR analysis). They process fibrous and soft products quickly and gently to the required analytical fineness of about 0.5 mm. The mills are ideally suited for grinding various types of non-fatty food. They are equipped with a rotor and grinding ring with sieve insert. The high-revolution speed of up to 14,000 min^{-1} and the grinding geometry of the rotor and grinding chamber generate an air stream which carries the sample through

the integrated cyclone into the sample bottle. This helps to avoid cross-contaminations. The cyclone provides additional cooling of the sample and the grinding tools. This prevents loss of moisture and thermal degradation ensuring preservation of the sample properties to be determined. The ground material is separated in the cyclone and collected in a sample bottle for complete recovery. The rotor speed can be adjusted in three steps allowing for perfect adaptation to sample requirements.

Tips and techniques:

- Quick exchange of sample bottles for increased throughput of samples.

- No cross-contamination and low cleaning effort required.

- As the grinding principle of cyclone mills has impact and friction, the machine should not be used for fatty sample materials like oil seeds.

3.1.3. Rotor beater mills

Rotor beater mills are used for the preliminary and fine-size reduction of soft, medium-hard, and brittle materials with a maximum feed size of 25 mm. The final fineness is determined by the aperture size of the exchangeable ring sieves (0.08–10 mm). A fineness down to 50 μm and below, depending on the properties of the sample material, may be achieved. Size reduction in the rotor beater mill is effected by impact and shearing forces between the vertical rotor and the ring sieve. To achieve an additional size reduction effect through impact, a 180°-grinding insert may be used for harder materials. The revolution speed is adjustable between 3000 and 10,000 min^{-1}. For larger sample quantities, a vibratory feeder can be used for automated feeding. In contrast to ultra centrifugal mills and cyclone mills, rotor beater mills are also suitable for grinding large sample amounts up to 30 L in one step.

Tips and techniques:

- A higher speed generates a higher throughput and less frictional heat.

- For temperature-sensitive materials, the use of a distance rotor is recommended. The larger grinding gap ensures a reduction of frictional heat.

- A cyclone is available which also reduces the heat build-up by discharging ground particles quickly out of the grinding chamber and generating a cooling air flow.

3.2. Knife mills

Typical applications: fresh meat, herbs, milk powder, fresh bacon, convenience food, cereal bars, soy beans, cakes, fresh fish, salad, cabbage, raisins, tomatoes, apples, fresh vegetables, sweets, jelly bears, bread, cheese, liver, fruits, chocolates, salami, soups, potatoes, cookies, waffles, ground beef, berries, nuts, seeds, boiled eggs, and so on.

Knife mills are suitable for the size reduction and homogenization of samples with a high fat, oil, or water content. They are frequently used in food control laboratories. The larger knife mills homogenize sample amounts up to 4500 mL, and are therefore the only mills which can homogenize a whole pizza or a loaf of bread in one batch. The speed range of the knife mills is flexible and allows for optimum adaption to the specific sample properties. When the mills are operated in reverse mode, the blunt end of the blades hits the sample with impact and crushes it (instead of cutting in a forward mode). A wide range of accessories are available: different knives and lids, containers of polypropylene, polycarbonate, stainless steel, and glass. Except for the polypropylene-grinding container, all containers can be autoclaved.

Tips and techniques:

- By using a gravity lid, the volume of the container is reduced and automatically adapted to the sample amount.

- For samples with a high liquid content, gravity lids with overflow channels are best suited. The liquid of the sample, which ascends the container walls, is returned to the center of the container for further homogenization.

- For heavy-metal-free grinding processes, neutral-to-analysis knives are available.

- By grinding in two or more steps (e.g., by changing from reverse to forward mode or by increasing the speed step by step), better grinding results may be achieved than by grinding in just one step.

- Always use the lid with two sealings when grinding wet samples in the larger-sized knife mills. Very wet samples must not be ground with maximum speed from beginning on, as the sample may splash out of the grinding container despite the sealings.

3.3. Ball mills

Typical applications include chocolate cream, spices, herbs, tea, olive pulp, lactose powder, egg shells, jelly bears, chitosan powder, liver, vanilla pods, berries, cookies, tobacco, chewing gum, wheat, waffles, frozen fish, seeds, and so on.

Ball mills are frequently used for the pulverization of hard-brittle materials. A crucial advantage of ball mills is their great versatility. Grinding jars and balls are available in various sizes and materials, for example, agate or ceramics such as zirconium oxide. This is important if the sample is analyzed for heavy metals. The grinding tools for ball mills consist of a grinding jar and grinding balls made of the same material. Mixer mills and cryo mills are the most widely used ball mills for homogenizing food samples.

Tips and techniques of ball mills in general:

- The following rule of thumb applies for the jar filling (dry grinding): one-third is filled with balls, one-third filled with sample material; thus, enough free space is left for ball movement. Also, this filling level ensures better grinding efficiency and less wear.

- When choosing the ball size, the feed size of the sample must be considered. For example, 30-mm grinding balls are suitable to grind particles of up to 10 mm.

3.4. Mixer mills

Mixer mills are suitable for grinding small sample quantities of up to 20 mL. The grinding jars perform radial oscillations in a horizontal position with a maximum frequency of 30 Hz. Size reduction is effected through impact forces, allowing for a final fineness down to $d_{90} = 5$ µm, depending on the sample properties. Grinding jars for mixer mills usually have a size range from 1.5 to 50 mL. When they are equipped with a screw-top lid, they are suitable for wet grinding. Another option is the use of different adapters which hold up to 20 × 2 or 10 × 5 mL reaction vials or 8 × 50 mL conical centrifuge tubes. Cryo mills are mixer mills specially designed for cryogenic grinding. They will be discussed later in the subchapter of cryogenic grinding.

Tips and techniques:

- If only one jar is filled with sample, the empty one should still be clamped to the second grinding station for balancing reasons.

- Closed grinding jars of steel can be cooled in liquid nitrogen to embrittle the sample material. Take care to fill in sample and grinding balls before cooling! Liquid nitrogen or dry ice must never be filled into the grinding jar—this would lead to overpressure inside the jar.

- Don't use grinding jars with mixed materials (e.g., steel jar with zirconium inlet) as the materials may react differently to very cold temperatures, leading to stress inside the jars and possibly to damages of the inlet.

3.5. Cutting mills

Typical applications include roots, tea, corn, freeze-dried fish, bones, mushrooms, spices, orange peel, sugar beet pellets, shea nuts, sugar cane, herbs, potatoes, lumps of cocoa butter, and so on.

Cutting mills are used for preliminary size reduction of soft, medium-hard, or fibrous materials such as roots, nut shells, or bones. Depending on the model, the revolution speed of the cutting mill is fixed or variable up to 3000 min⁻¹. The achievable grind size depends on the aperture size of the exchangeable bottom sieve (ranging from 0.25 to 20 mm) and the breaking properties of the sample material. Three types of rotors are available to find the best way to crush a specific sample: a parallel section rotor, acting like an axe, which is especially suitable for soft, elastic, and fibrous materials; a six-disc rotor with replaceable and reversible tungsten carbide plates, acting like a shredder, which is especially suitable for medium-hard materials; and finally, the V-rotor, acting like scissors, which is especially suitable for tough, soft, and fibrous material, improving the grinding process and sample discharge.

Tips and techniques:

- A cyclone helps to discharge the sample from the grinding chamber much quicker and leads to a cooling effect thanks to the generated air stream.

- Choose the most suitable rotor for a sample. The rotors either act like a shredder, an axe, or like scissors.

4. Application examples: homogenization of food

4.1. Fat content in sausages (knife mill)

Sausages often contain large fatty particles. They need to be thoroughly homogenized to ensure reliable analysis results. If the few grams required for fat content analysis were picked randomly from the sample, this would result in increased standard deviations of the analysis results. Two hundred grams of sausages was ground in two steps. After the sausages were cut manually into pieces of approximately 20 mm, a first grinding cycle was carried out in a knife mill at a revolution speed of 10,000 min^{-1} using a knife with serrated blades. The sample was cut to pieces smaller than 5 mm in only 15 s. The serrated blades help to tear the fibrous meat. A part sample was taken directly for fat analysis. The remaining sample was pulverized under cryogenic conditions. For this purpose, the sample was mixed with dry ice snow (with a ratio of 1:2) after the first grinding step and the mixture was then filled into a grinding container of stainless steel. Using a full metal knife and a lid specifically designed for cryogenic grinding, the sample was pulverized by grinding at 4,000 min^{-1} for 3 × 10 s (**Figure 5**).

Both the coarse and the homogenized samples were analyzed for their fat content five times by microwave-induced drying combined with nuclear magnetic resonance (NMR) spectroscopy. For each measurement, 4 g of sample was dried for 2.5 min and analyzed within 1 min. The fat content of the independent samples of the coarse sausage varies more than that of the finely ground samples. The fat content of the coarser fraction was measured in a range from 14.85 to 17.12% with a standard deviation (SD) of 0.88%. The SD was reduced by more than a factor of 10–0.07% in the homogenized sample (**Figure 6**), with a fat content ranging from 15.84 to 16.02% (relative SD reduced from 5.63 to 0.45%).

Figure 5. Homogenization of sausages; from left to right: original sample; pre-cut sample with large fatty parts; sample ground to <5 mm; pulverized sample of <300 μm.
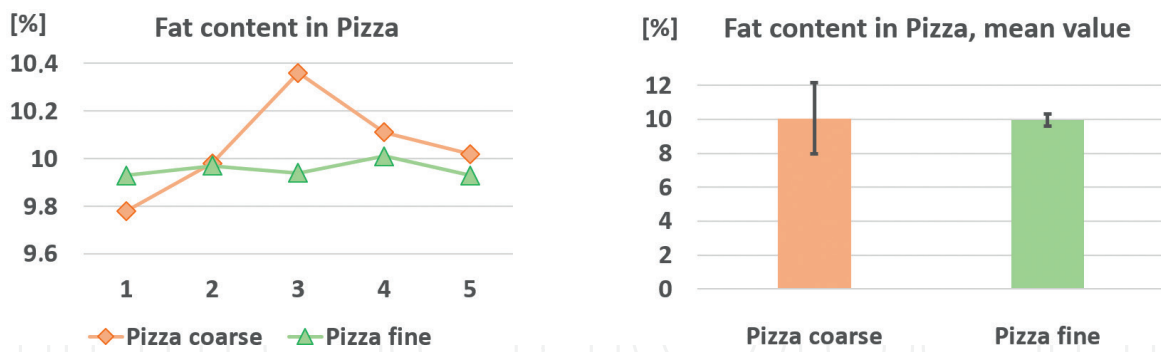
Figure 6. Left: fat content varies in coarse sausage samples, but is stable in fine-ground samples; right: the mean values of five samples of each of the relative standard deviations of the fat content in sausages were reduced from 5.63 to 0.45% by fine grinding.

4.2. NIR analysis of wheat samples (cyclone mill)

NIR is a common analytical method for the determination of protein content, moisture, fat, and ash in one run. Therefore, it is used whenever a high-sample throughput and great flexibility are required. A much-discussed issue is the necessity of sample preparation. What are the advantages of sample preparation before NIR analysis? The penetration depth of NIR radiation is 1 mm maximum, so everything that lies beneath cannot be detected. That is not a problem if the sample is completely homogeneous, but if a sample consists of different layers, like grains or seeds, then only the layers down to 1 mm are analyzed and are consequently overrepresented in the measurement results. To demonstrate this effect, the different properties of ground and unground wheat samples were analyzed with NIR [5]. The samples were analyzed 10 times, and the spectrometer was refilled for every measurement. The samples were pulverized in a cyclone mill. Cyclone mills are suitable for processing a variety of different materials which is ideal for NIR analysis requirements. The results for wheat show a large discrepancy between ground and unground sample, especially regarding the ash and fiber content (**Table 1**). As explained above, only the surface of the unground wheat is analyzed resulting in an overrepresentation of the kernel shell. Meaningful and reliable analysis results are guaranteed only by sample homogenization.

	Ash	Moisture	Fiber	Fat	Protein
Ground wheat					
Average [%]	2.80	9.68	1.10	1.17	9.02
Standard deviation [%]	0.03	0.09	0.05	0.03	0.07
Unground wheat					
Average [%]	0.10	9.80	6.90	1.38	8.46
Standard deviation [%]	0.10	0.25	0.62	0.16	0.45

Table 1. The analysis of wheat shows a difference in the ash and fiber content of the ground and unground sample.

4.3. Detection of mycotoxins in nuts (cutting mill and ultra centrifugal mill)

Mycotoxins are natural metabolism products of molds which have a toxic effect on humans and animals. Some types of food show an increased risk of mycotoxin release due to fungal infestation, especially when food is stored too long and in an unsuitable way. Fungal infestation usually occurs in nests, a random sample taken from the bulk must be sufficiently large to allow for the detection of contaminants. The first step is the preliminary size reduction of a representative amount of 1–2 kg per ton of nuts with a cutting mill to particles of <3 mm by using a bottom sieve of 4 mm [6]. It is important to use a six-disc rotor, as the shells of the nuts are too hard for the cutting effect of the other rotors. The subsequent fine-size reduction is ideally carried out with an ultra centrifugal mill. For the processing of hazelnuts, the use of distance sieves is recommended. As mycotoxins are lipophilic, the grinding process should be as gentle as possible to avoid the release of fat from the sample. A fineness of 300 µm (**Figure 7**) is sufficient for the subsequent extraction of the mycotoxins and for high-performance liquid chromatography (HPLC) analysis.

Figure 7. Homogenization of nuts; from left to right: original sample; pulverized sample of <300 µm.

4.4. Detection of amino acids in fatty, fresh bacon (knife mill)

Tough sample materials like fatty, inhomogeneous, streaky bacon pose a challenge to the homogenization process prior to analysis [7]. If larger parts of the rind or skin remain uncut, the sample is not homogeneous and the analysis may yield false results. Knife mills have proven to be best suited for thoroughly homogenizing meat samples (**Figure 8**). A strong motor to make use of the full cutting capacity of the blades is beneficial. Serrated blade knifes are ideally suitable for homogenizing tough meat samples in a very short time, as an additional tearing effect facilitates size reduction of the meat fibers. Short grinding times ensure low heat build-up. To obtain a thoroughly homogenized sample (at room-temperature conditions), the grinding process may require two or three steps. Two hundred and fifty grams of pork shoulder is processed in a knife mill with interval mode at a revolution speed of 3000 min^{-1}, using a serrated blade knife for 30 s. The first step is followed by two cycles of 30 s, each at 7000 min^{-1}. The best homogenization of the sample is achieved after another 30 s at 10,000 min^{-1}. The sample would bounce too much if the maximum speed was selected right from the start. Nonetheless, full speed is required at some point to achieve the best possible results. It is also important to use a

standard lid, as other lid types might put too much pressure on the sample. The sample parts sticking to the grinding container wall above the blades need to be removed from time to time and returned to the grinding process. The sample is now ready for the detection of amino acids via color reaction with iTAG solution.

Figure 8. Homogenization of bacon; from left to right: original sample; homogenized sample.

4.5. Detection of polychlorinated biphenyls in fish (cutting mill)

The homogenization of fish is a challenge; scales, skin, and bones are fairly resistant to size reduction so that the sample still contains larger pieces after grinding in most mills (e.g., fresh fish in knife mills). A high fat content of the fish makes the process more difficult, as fatty particles stick together to form large lumps which block the mill and keep the sample inhomogeneous. Freeze drying of the fish and further milling in a cutting mill helps to solve the problem. 125 g (four fishes, pre-cut once) of carp or turbot were pulverized in a cutting mill at a revolution speed of 3000 min^{-1}, using a V-rotor which also cuts the scarp and fish bones. The use of a cyclone cools the sample. After 2 min of grinding with a 1-mm bottom sieve, the fish is ground to 1-mm particles without significant heat build-up (**Figure 9**). The sample is now ready for extraction and subsequent gas chromatography.

Figure 9. Homogenization of fish; from left to right: original sample, sample ground to <1 mm.

4.6. Pyrrolizidine alkaloids in tea (ultra centrifugal mill)

The group of pyrrolizidine alkaloids comprises 500 chemical compounds which are mostly found in composite flowers, borage family, and leguminous plants. Dried chamomile flowers were processed with the following parameters: a 25-g sample with a maximum particle size of

5 mm was pulverized at a revolution speed of 18,000 min^{-1} in an ultra centrifugal mill using a 0.2-mm ring sieve. After 2 min, the complete sample was ground to a final fineness of <100 μm (**Figure 10**). The use of a cyclone ensures continuous material discharge and cooling of the sample. Thus, the characteristics of the heat-sensitive pyrrolizidine alkaloids are preserved during sample preparation and can be detected by SPE-LC-MS/MS.

Figure 10. Homogenization of chamomile; left to right: original sample, sample ground to <100 μm.

4.7. Ginsenoide in ginseng (mixer mill)

Ginseng has been known for many years in traditional Chinese medicine to have beneficial health effects such as boosting immune reaction and supporting the cardiovascular system. A certain class of chemical substances, such as ginseng saponins, seems to be responsible for the beneficial effects. Therefore, analyzing the composition and content of these substances is of great interest. Small amounts of ginseng roots can be pulverized in mixer mills provided they are smaller than 8 mm. Larger sample pieces must be cut first, for example, by using a cutting mill with a parallel section rotor. 17 mL of pre-cut ginseng particles was pulverized in a mixer mill in a 50-mL stainless steel grinding jar. Fifteen grinding balls with 10-mm diameter were used. After 4 min at a frequency of 30 Hz, a final fineness below 100 μm was achieved (**Figure 11**). The sample was now ready for extraction and subsequent HPLC analysis.

Figure 11. Homogenization of ginseng; left to right: original sample, sample ground to <8 mm, sample ground to <100 μm.

4.8. Mineral determination in large quantities of salt (rotor beater mill)

Rock salt and sea salt not only consist of sodium chloride but may also contain other minerals and silicates, depending on the mining area and method. To analyze the composition of salt, the sample needs to be sufficiently homogenized, considering that larger lumps of rock salt are usually very inhomogeneous. The element concentrations in salt are usually very low so

that it is frequently necessary to process amounts in the kilogram range. In principle, a cutting mill could cope with large quantities but the wear would be much greater than in a rotor beater mill, as the cutting bars of the cutting mill are not designed to process large amounts of abrasive materials like salt. With a rotor beater mill, charges of several kilograms can be pulverized easily. Size reduction of the sample is effected by impact and shearing. A distance rotor is used to reduce frictional heat. Thanks to a 5-L collecting vessel, 5 kg of sample with a feed size up to 10 mm is pulverized in one run at a revolution speed of 10,000 min^{-1}. The complete sample is pulverized to less than 200 μm in 6 min (**Figure 12**) and can be analyzed by colorimetric methods or titration.
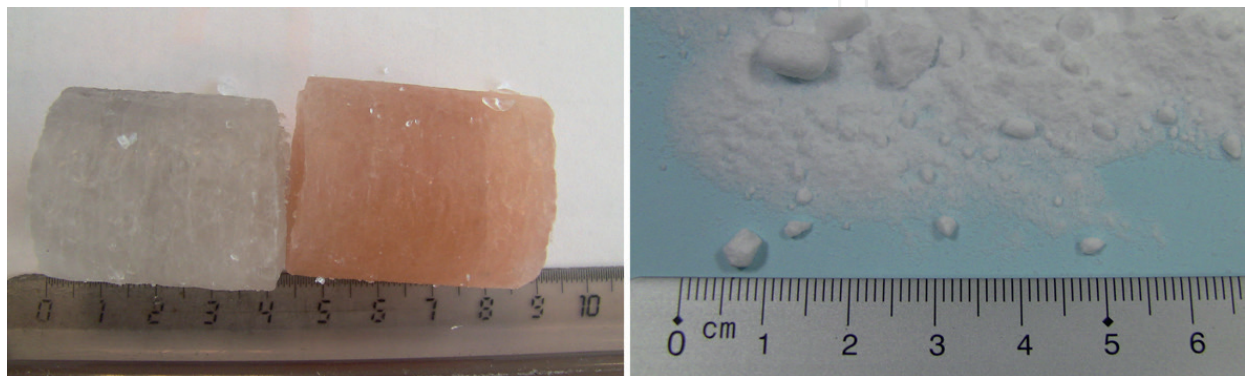

Figure 12. Homogenization of rock salt; left to right: original sample, sample ground to <200 μm.

4.9. Vitamin C analytics in hard candy (knife mill)

Confectionery occurs in very different textures: it can be hard, sticky, greasy, or moist and is frequently inhomogeneous. For HPLC analysis, which is used to detect the content of vitamin C, for example, in hard candy, a particle size distribution between 0.5 and 0.75 mm is ideal. A typical homogenization process in a knife mill involves 100 g of hard candy which is first roughly ground for a few seconds in reverse mode with the blunt side of the knife [8]. The following step involves operation in forward mode with intervals for another 15 s at a revolution speed of 4000 min^{-1}. Further pulverization to a size below 0.5 mm is achieved by grinding for 6–12 s at a revolution speed of 6000 min^{-1} (**Figure 13**). This step-by-step procedure prevents the sample—which has a high sugar and starch syrup content—from sticking to the knife as is often the case in household mixers.

Figure 13. Homogenization of candy; left to right: original sample, sample ground to <500 μm.

4.10. Detection of genetically modified organism in soy beans (knife mill)

Polymerase chain reaction (PCR) is used to detect genetically modified organisms (GMOs) in food. Prior to PCR, the sample must be homogenized. Attention should be paid to sampling and obtaining a representative part sample to ensure meaningful and sensitive GMO testing. From a 20-t bulk of soy beans, a laboratory sample of about 2.5 kg is extracted. For the detection of GMOs a smaller analysis sample, approx. 1000 g in case of corn or soy beans, is extracted from the laboratory sample and thoroughly homogenized in a knife mill. For PCR analysis, only 2 mg of sample material is required. The homogenization step ensures that this 2 mg is a representative of the whole sample. Grainy food like soy beans is processed in a steel container at a revolution speed of 10,000 min^{-1}. With batches of 4 × 250 g, grind sizes below 0.5 mm are obtained within 30 s (**Figure 14**).

Figure 14. Homogenization of soy beans; left to right: original sample, sample ground to <500 μm.

4.11. Further applications: food homogenized at room temperatures

In the following, more application examples for homogenization of food samples at room temperature are given (**Table 2, Figure 15**), before putting a focus on cryogenic grinding in the next section.

Sample	Parameters and accessories	Size reduction	Remark
20 g nuts[1]	10,000 min^{-1}, 10 s; grinding container stainless steel	15 to 0.5 mm	High fat content may lead to blockages of sieves in rotor mills
200 g lemons[1]	8000 min^{-1}, 10 s; gravity lid with overflow channels	80 mm to paste	High water content and large particle size: milling only in a knife mill possible
160 g pie [1]	10 s 4000 min^{-1}, 10 s 8000 min^{-1}	30 mm to paste	Starting with short intervals at the set speed helps to avoid material sticking on walls of grinding container
280 g lasagna[1]	10 s 4000 min^{-1}, 20 s 8000 min^{-1}	80 mm to paste	
500 g bread[1]	1 min 4000 min^{-1}, knife with titanium-niob coated blades	160 to 1.5 mm	Heavy metal determination: knife with titanium-niob coated blades was used
100 g dried pear[1]	15 s 4000 min^{-1}, 15 s 7000 min^{-1}	50 to 1 mm	Homogenization of sticky material
200 g hard cheese[1]	10 s 2000 min^{-1}, 10 s 6000 min^{-1}	20 to 1.5 mm	Finer particles could be obtained under cryogenic conditions

Sample	Parameters and accessories	Size reduction	Remark
800 g soup[1]	30 s 4000 min^{-1}, with interval	50 mm to paste	Double-sealed lid for liquid samples, interval mode improves sample mixing
700 g pizza[1]	90 s 2000 min^{-1}, with interval	50 to 3 mm	Cryogenic grinding can achieve further homogenization
150 g ginger[1]	35 s 4000 min^{-1} reverse mode	30 to 0.8 mm	Reverse mode helps to avoid wear of blades when cutting tough material
5 eggs[1]	10 s 10000 min^{-1}	70 mm to paste	Very fast homogenization
100 g field bean[2]	12 tooth rotor, distance sieve 1 mm, 60 s, 18000 min^{-1}	15 to 0.5 mm	To avoid warming, the sample is filled into the mill slowly but continuously. The distance sieve is used to reduce heat.
150 g gelatin[2]	12 tooth rotor, distance sieve 1 and 0.35 mm, 45 and 120 s, 18000 min^{-1}, cyclone	70 to 0.5 mm	Distance sieve to avoid warming, slow feeding required, cyclone helps to cool sample and improve sample discharge
1200 g salt[2]	12 tooth rotor, distance sieve 0.08 mm, 10 min, 18000 min^{-1}, cyclone and vibratory feeder	1 mm to 15 μm	Sample is hygroscopic and may stick; check sample discharge from time to time, vibratory feeder facilitates feeding of larger quantities
50 g green coffee[2]	12 tooth rotor, distance sieve 0.75 mm, 3 min, 18000 min^{-1}, cyclone	15 to 0.75 mm	Distance sieve and cyclone reduce heat and fat release. Sieves with small aperture sizes may be blocked due to fat release.
150 g corncob[3]	Parallel section rotor, bottom sieve 4 mm, 1500 min^{-1}, 20 s; & 12 tooth rotor, ring sieve 0.5 mm, 20 s, 18,000 min^{-1}	150 mm to 400 μm	Grinding in two steps as initial sample is too large for direct feeding into ultra centrifugal mill; required final fineness achieved efficiently in ultra centrifugal mill
50 g viola roots[3]	Six-disc rotor, bottom sieve 4 mm, 1500 min^{-1}, 20 s & 12 tooth rotor, ring sieve 0.5 mm, 15 s, 18,000 min^{-1}	100 mm to 200 μm	Sample is too hard for manual pre-crushing, fine grinding in ultra centrifugal mill as second step yields very fine material
5 kg tea[4]	V-rotor, 0.25 mm bottom sieve, 3000 min^{-1}, 25 min	6 cm to 200 μm	Less warming of the sample compared to ultra centrifugal mill but same fineness and time
10 pieces, gelatin blocks[4]	V-rotor, 6 mm bottom sieve, 3000 min^{-1}, 10 s, cyclone	8 × 5 × 1 cm to 6 mm	The cyclone is used to increase sample discharge from grinding chamber (very light material)
10 kg oat[4]	Parallel section rotor, 6 mm bottom sieve, 700 min^{-1}, 60 s, cyclone	6 to 3 mm	Reduction of speed increases obtained particles size, fine fraction is reduced
50 g mushrooms[4]	Parallel section rotor, 6 mm bottom sieve, 1500 min^{-1}, 10 s	30 to 4 mm	Sample was ground piece by piece, high-coarse particle content required
2 l manioc[5]	0.25 mm 360° sieve, cyclone, feeder, 10,000 min^{-1}, 11 min	2 mm to 200 μm	Vibratory feeder for larger quantities
20 kg roasted milk with sugar[5]	2 mm 360° sieve, cyclone and feeder, 30 l receptacle, 10,000 min^{-1}, 38 min	3 to 1 mm	Distance sieve reduces sticking of sample; 30 l receptacle required for large sample quantity

Sample	Parameters and accessories	Size reduction	Remark
2 kg herbs[5]	0.08 mm 360° sieve, cyclone, feeder, 30 l receptacle, 10000 min^{-1}, 80 min	15 mm to 120 μm	Vibratory feeder for large quantities, 80 min to process this large sample quantity to a fineness of 120 μm
30 g corn[6]	0.5 mm sieve, 14,000 min^{-1}, 15 s	10 to 0.3 mm	Quick and contamination-free grinding of non-fatty samples, high-sample throughput
100 g barley[6]	1 mm sieve, 14,000 min^{-1}, 10 s	10 to 1 mm	
50 g dry noodles[6]	2 mm sieve, 14,000 min^{-1}, 20 s	15 to 0.75 mm	

[1]Knife mill.
[2]Ultra centrifugal mill.
[3]Cutting mill and ultra centrifugal mill.
[4]Cutting mill.
[5]Rotor beater mill.
[6]Cyclone mill.

Table 2. Application examples of food homogenized at room temperature.

Figure 15. Food samples which can be pulverized at room temperature; first row from left to right: pistachios, lemon, pie, lasagna, bread, pears, cheese, soup; second row from left to right: ginger, beans, green coffee, corncob, viola root, maize, barley, noodles; third row from left to right: manioc, roasted milk and sugar, herbs, block of gelatin, salt, pizza, nuts, sausages.

5. Special application: cryogenic grinding of food samples

Most sample materials can be ground to the required analytical fineness at room temperature. However, there are limits, for example, when even a small temperature increase affects the

sample in a negative way, or when the material is very elastic and will only be deformed. Moreover, food samples, which are fatty or sticky, may block the mill. Cryogenic grinding is the best way to pulverize food samples when they are sticky, fatty, semi-liquid samples (e.g., cheese, raisins, wine gum, or marzipan), and simply clump together when ground at room temperature. In a cryogenic-grinding process, the samples don't clump and are effectively homogenized. Under cryogenic conditions, the loss of volatile ingredients like alcohol can be limited or residues of softeners, which migrate from plastic wrappings into fatty food like meat, are preserved. Such ingredients would escape when the sample is warmed during grinding. Furthermore, cold milling preserves the original structures of vitamins or proteins. Cryogenic grinding is carried out with grinding aids such as liquid nitrogen LN_2 (−196°C) or dry ice (solid CO_2; −78°C) which embrittle the sample and make it break more easily. In this section, the special requirements for cryogenic grinding in different mills will be discussed as well as which other aspects need to be taken into consideration (**Table 3**). Basically, all rules and recommendations described for grinding at room temperature must be observed for cryogenic grinding, too.

5.1. Cryogenic grinding in mixer mills

It is important to fill the jar first with the grinding ball(s) and with the sample and close it tightly before embrittling. Care must be taken that no LN_2 is enclosed in the grinding jars because the evaporation of the LN_2 would result in a considerable pressure increase inside the grinding jar. The closed grinding jars, and thus the sample, are embrittled in a LN_2 bath for 2–3 min. Suitable grinding jars for cryogenic grinding are made of steel or PTFE; it is not recommended to use jars made of different materials (e.g., steel jar with lining of zirconium oxide). This is important, as two different materials may react differently to extreme temperatures of −196°C which may lead to damages of the jar. Single-use vials of 1.5, 2, and 5 mL are also available for cryogenic grinding. Due to the high-energy input and the resulting frictional heat, the grinding process should not take longer than 2 min to prevent the sample from warming up and to preserve its breaking properties. If longer grinding times are required, these should be interrupted by intermediate cooling of the closed grinding jars.

5.2. Cryogenic grinding in cryo mills

Cryo mills offer the advantage of continuous cooling of the grinding jar with LN_2, reducing the temperature of jar and sample to −196°C within minutes. Thus, a consistent temperature of −196°C is guaranteed even for long grinding times without the need for intermediate cooling breaks. Moreover, care should be taken that the user comes at no point into contact with LN_2. An automatic pre-cooling function ensures that the grinding process does not start before a temperature of −196°C is reached and maintained. For heavy-metal-free grinding, a zirconium oxide grinding jar should be used. Further suitable materials are stainless steel or single-use vials (1.5 or 2 mL). Just like in mixer mills, embrittlement of the sample occurs indirectly as the sample is enclosed in the grinding jar.

Mill	Feed size and max feed quantity (both depending on sample material)	Remark
Mixer mill	<8 mm 2 × 20 mL	• Sample is placed in leak-free grinding jar of steel or PTFE and embrittled before grinding, LN_2 preferred over dry ice • Intermediate cooling may be required
Cryo mill	<8 mm 1 × 20 mL	• Continuous grinding at −196°C with LN_2 • User comes at no point in contact with LN_2 • Zirconium oxide grinding jar available for cryogenic grinding
Ultra centrifugal mill	<10 mm 4000 mL	• Embrittlement with dry ice or LN_2 • Dry ice preferred if sample material is <1 mm or has low thermal capacity • Use of cyclone mandatory
Knife mill	<40 mm 2000 mL	• Embrittlement with dry ice • Dry ice cools sample during grinding • Use of full metal knife, grinding container of stainless steel and specific lid mandatory
Cutting mill	<80 mm 4000 mL	• Cryogenic grinding with dry ice or LN_2 • Use of six-disc rotor and cyclone mandatory • Bottom sieves 2–20 mm suitable

Table 3. Overview of mills suitable for cryogenic grinding.

5.3. Cryogenic grinding in ultra centrifugal mills

Ultra centrifugal mills accept larger sample volumes than mixer mills. The sample is directly immersed into a container filled with LN_2 before being continuously but slowly fed to the hopper of the mill with a steel spoon. When using dry ice as grinding aid, this needs to be mixed with the sample (one part sample, two parts dry ice) and the entire mixture is then pulverized in the mill. Using a cassette in combination with a cyclone is recommended for cryogenic grinding to ensure that the evaporating cooling agent is completely discharged during the grinding process. The use of dry ice rather than LN_2 should be preferred if the sample is already smaller than 1 mm, as the transfer of a dry ice-sample mixture to the mill is much easier than fishing the sample with a spoon from the LN_2 bath. Also, if the sample has a low thermal capacity, dry ice is also preferable as it cools the sample during grinding.

5.4. Cryogenic grinding in knife mills

Sticky and tough food samples such as cheese, raisins, wine gum, or marzipan are perfectly homogenized in a knife mill. The use of LN_2 is not recommended as the knife mills are not designed for temperatures as low as −196°C. Even chocolate, which simply becomes paste-like when processed at room temperature, can be successfully pulverized cryogenically. The sample is mixed with dry ice in a ratio of 1:2; after a few minutes, it is thoroughly cooled and the grinding process starts. The dry ice keeps the sample cool all the time. Care should be taken not to use any plastic accessories when carrying out cryogenic grinding in the knife mills as these could be damaged during the process. Suitable accessories include a grinding container of stainless steel, a full metal knife, and a lid with aperture to allow evaporation of the gaseous carbon dioxide.

5.5. Cryogenic grinding in cutting mills

Cutting mills are particularly suitable for processing larger feed sizes than ultra centrifugal mills or knife mills. Both the use of LN_2 and dry ice are possible (see Section "Cryogenic grinding with ultra centrifugal mills" for advantages of using dry ice). The embrittled sample material is rather hard; therefore, the use of the six-disc rotor is recommended as it works more like a shredder. It is also suitable to cut heterogeneous samples such as frozen chicken parts including bones.

5.6. Cryogenic applications: food homogenized at low temperatures

Table 4 provides an overview of samples which are best ground under cryogenic conditions (**Figure 16**).

Sample	Parameters and accessories	Size reduction	Remark
10 jelly bears[1]	1 min, 30 Hz	20 mm to 300 μm	Tongs to transfer the grinding jars from LN_2 bath into mill. Grinding in 50 mL with grinding ball 25 mm grinding jar (both stainless steel)
20 g chewing gum[1]	30 s, 30 Hz	15 mm to 500 μm	
10 g liver[1]	2 min, 30 Hz	6 mm to 400 μm	
10 g cookies[1]	1 min, 30 Hz	12 mm to 300 μm	
3 g vanilla pod[1]	20 s, 30 Hz	10 mm to 500 μm	
2 g cherries[2]	10 s, 30 Hz	15 mm to 600 μm	Food samples: pre-cooling of appr. 5 min is typical. Usually grinding is done in 50-mL grinding jar with grinding ball 25 mm (both stainless steel)
8 g pork[2]	3 min, 30 Hz	10 mm to 200 μm	
6 g licorice[2]	2 min, 30 Hz	10 mm to 300 μm	
9 g green coffee[2]	15 min, 30 Hz	10 mm to 150 μm	
5 g cheese[2]	2 min, 30 Hz	8 mm to 300 μm	
1 praline; liquid filling[2]	2 min, 30 Hz	10 mm to 400 μm	

Sample	Parameters and accessories	Size reduction	Remark
500 g wine gum[3]	40 s 2000 min^{-1} reverse; 20 s 4000 min^{-1} forward	20 to 0.8 mm	Grinding container stainless steel, full metal knife, cryo lid with aperture; dry ice. Pre-cutting in reverse mode reduces wear of blades. Intervals can help to improve sample mixing.
250 g grapes[3]	15 s 2000 min^{-1} reverse; 15 s 4000 min^{-1} forward	20 mm to 400 µm	
300 g block of marzipan[3]	20 s 2000 min^{-1} reverse; 20 s 4000 min^{-1} forward	40 mm to 800 µm	
400 g pure bacon[3]	45 s 2000 min^{-1} reverse; 30 s 4000 min^{-1} forward	30 to 1 mm	
800 g raisin[3]	45 s 2000 min^{-1} reverse	15 to 0.5 mm	
100 g cereals[4]	12 tooth rotor, ring sieve 0.5 mm, 3 min, 18000 min^{-1}	8 mm to 250 µm	Use of cyclone and LN$_2$
70 g nutritionals[4]	12 tooth rotor, ring sieve 0.12 mm, 5 min, 18,000 min^{-1}	2 mm to 100 µm	Use of cyclone and dry ice. Grinding in two steps using two different ring sieves is efficient if initial sample size is larger.
100 g dried apples[4]	12 tooth rotor, ring sieve 0.5 mm, 1 min, 18000 min^{-1}	5 mm to 250 µm	
15 g toffee candy[4]	12 tooth rotor, ring sieve 2 mm and 0.5 mm, 1 min, 18,000 min^{-1}	10 mm to 500 µm	
500 g block of chocolate[5]	Parallel section rotor, 4 mm bottom sieve, 700 min^{-1}, 60 s	40 to 4 mm	Use of cyclone and LN$_2$; reduced speed leads to less heat build-up
1 kg trout[5]	6-disc rotor, 20 mm bottom sieve, 700 min^{-1}, 60 s	200 to 20 mm	
500 g lump of cocoa butter[5]	six-disc rotor, 6 mm bottom sieve, 700 min^{-1}, 90 s	100 to 6 mm	
20 kg sweet potatoes[5]	6-disc rotor, 20 mm bottom sieve, 1500 min^{-1}, 15 min	100 to 20 mm	Use of cyclone and dry ice

[1]Mixer mill.

[2]Cryo mill.

[3]Knife mill.

[4]Ultra centrifugal mill.

[5]Cutting mill.

Table 4. Application examples of cryogenically homogenized food.

Figure 16. Food samples ground under cryogenic conditions; first row from left to right: jelly bears, chewing gum, fresh liver, cookies, vanilla pods, dried cherries, fresh meat; second row from left to right: licorice, cheese, praline with liquid core, wine gum, grapes, block of marzipan, bacon; third row from left to right: raisins, onions, bar of cereals, dried apples, block of chocolate (pre-ground), frozen fish (pre-ground), cocoa butter (pre-ground).

6. Conclusion

In this chapter, it was demonstrated by a wealth of application examples that sample preparation prior to any food analysis is an essential step of the quality control process as only fully homogenized samples provide reliable and reproducible analysis results. Due to the wide range of laboratory mills and accessories available, it is important to consider all aspects of the sample preparation process before selecting a suitable device to make this important step prior to sample analysis most efficient and reliable. Both the knowledge of the sample characteristics and the available types of mills and accessories enable the user to process these samples with a minimum of time and effort but with best possible results.

7. General remark

See more detailed information on our webpage www.retsch.com—the different application reports, brochures, "the sample 43," and the "Art of Milling" may be downloaded.

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