### Task:

**Application field:** Agriculture

**Material:** Horse Bone:
1) Horse Tibia;
2) Horse teeth bone
3) Horse Mandibula (lower chin bone)

**Feed size:** 30-50 mm (original sample)
approx. 1 - 8 mm after pre-cutting in Cutting Mill SM 200

**Feed quantity:** Complete sample in SM 200
5 g in CryoMill and Mixer Mill

**Material specification(s):** dry, brittle

**Customer requirements(s):** Fine enough for DNA-extraction (usually about < 50 µm)

**Subsequent analysis:** DNA Deoxyribo Nucleic Acid

### Solution

**Selected Instrument(s):** Cutting Mill SM 200
CryoMill
Mixer Mill MM 400

**Configuration(s) Item nos.:**
1 x MM 400, 100-240 V, 50/60 Hz
2 x Grinding jar, stainless steel, 25 ml, screw top design
2 x Grinding ball, stainless steel, 15 mm ø
1 x Cryo kit for cooling the grinding jars with liquid nitrogen optional:
1 x CryoMill, 100-240 V, 50/60 Hz
1 x Grinding jar, stainless steel, 25 ml
1 x Grinding ball, stainless steel, 20 mm ø
1 x Autofill with LN2 container and safety valve, 50 litres
1 x SM 200, 3/N~ 400 V, 50 Hz, cutting bars stainless steel
1 x 6-disc rotor, stainless steel, with reversible cutting tips of tungsten carbide
1 x Universal hopper with plastic plunger, for SM 200 / SM 300
1 x Bottom sieve, square holes, 8 mm, stainless steel

Please note: Other electrical versions of the instrument(s) are available with different item numbers.
| Parameter(s):          | SM 200: Revolution speed 1500 rpm  
|                       | MM 400: Frequency 30 Hz  
|                       | CryoMill: Frequency 25 Hz; 8 cycles |
| Time:                 | Sample 1, MM 400: 4x 2min. (+ approx. 1 min. intermediate cooling of the jar in liquid nitrogen)  
|                       | Sample 1, CryoMill: 8x 2 min. (automatic pre-cooling, 45s intermediate cooling)  
|                       | Sample 2, CryoMill: 8x 2 min. (automatic pre-cooling, 45s intermediate cooling) |
| Achieved result(s):   | See Horiba-reports (after using ultra-sonic, without ultra-sonic particles up to approx. 100 µm can be measured):  
|                       | Sample 1, MM 400: D90 = approx. 45 µm  
|                       | Sample 1, CryoMill: D90 = approx. 19 µm  
|                       | Sample 2, CryoMill: D90 = approx. 7 µm  
|                       | Sample 3, CryoMill: D90 = approx. 10 µm |
Remark(s): Pre-cutting of all samples in the Cutting Mill SM 200 (with hinged hopper) with a 8 mm bottom sieve. Sample could also be cut manually down to < 10 mm.

Sample 1, MM 400, test 1:
Grinding of 5 g sample with the Mixer Mill MM 400 in a 25 ml grinding jar with 1x 20 mm grinding ball of stainless steel (without cooling the grinding jar in liquid nitrogen). The material starts caking and agglomerating (sticking to the grinding jar wall and the ball).

Sample 1, MM 400, test 2:
Grinding of 5 g sample with the Mixer Mill MM 400 in a 25 ml grinding jar with 1x 15 mm grinding ball of stainless steel. In order to improve the breaking properties of the material and reduce the agglomeration and caking effects the filled grinding jar has been cooled in liquid nitrogen (in KryoKit). After every 1:30 or 2 min. grinding time the grinding jar should be cooled again in liquid nitrogen until it stops boiling. After 4x 2 min. the particle size has been measured. Still a little bit of sample is caking and agglomerating.

Sample 1, CryoMill:
Grinding of 5 g sample with the CryoMill in a 25 ml grinding jar with 1x 20 mm grinding ball of stainless steel. Due to the fact that the sample is cooled/pre-embrittled continously in the CryoMill a higher fineness can be achieved. All parameters can easily be adjusted at the machine. The user has no direct contact with liquid nitrogen. After 8 cycles of 2 min each the particle size has been measured with the Horiba LA-950.

Sample 2, CryoMill:
Grinding of 5 g sample with the CryoMill in a 25 ml grinding jar with 1x 20 mm grinding ball of stainless steel. Due to the fact that the sample is cooled/pre-embrittled continously in the CryoMill a higher fineness can be achieved. All parameters can easily be adjusted at the machine. The user has no direct contact with liquid nitrogen. After 8 cycles of 2 min each the particle size has been measured with the Horiba LA-950.

Sample 3, CryoMill:
Grinding of 5 g sample with the CryoMill in a 25 ml grinding jar with 1x 20 mm grinding ball of stainless steel. Due to the fact that the sample is cooled/pre-embrittled continously in the CryoMill a higher fineness can be achieved. All parameters can easily be adjusted at the machine. The user has no direct contact with liquid nitrogen. After 8 cycles of 2 min each the particle size has been measured with the Horiba LA-950.

The application report is based solely on the processing of the available sample material in the indicated amount. No legal claims shall be derived from this test report. Subject to technical modification and errors. © Retsch GmbH - www.retsch.com - lab@retsch.com
Without using ultra-sonic during measuring the particle size some agglomerates up to 100 µm are noticeable. After using ultra-sonic the agglomerates are destroyed and the "real" particle size could be measured (see "Achieved results")

**Recommendation:** The CryoMill and the Mixer Mill MM 400 are suitable to grind the sample materials under the above mentioned conditions.
Pictures of the sample:

Picture 1: Sample 1

Picture 2: Sample 1 after pre-cutting in SM 200

Picture 3: Sample 2

Picture 4: Sample 2 after pre-cutting in SM 200

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Laser Scattering Particle Size Distribution Analyzer LA-950

ID# : 201208071514837
Probenname : 13922_Horse Tibia MM400_25mlss_15mmball_4x2min
Datenbezeichnung : 13922_Horse talia_MM400_25mlss_15mmball_4x2min
Quelle : 
Transmission (R) : 88.2(\%) 
Transmission (B) : 86.4(\%)
Zirkulationsgeschwindigkeit : 6
Rührgeschwindigkeit : 01:00 (7)
Art der Verteilung : Volumen

D10 : 4.54043(\µm) 
Median : 12.40756(\µm) 
D90 : 45.22232(\µm)

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Laser Scattering Particle Size Distribution Analyzer LA-950

ID# : 201208080900838
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Datenbezeichnung : 13922_Horse Tibia_CryoMill_25mlss_20mmball_8x2min
Quelle :
Transmission (R) : 87.4(%) 
Transmission (B) : 82.7(%) 
Zirkulationsgeschwindigkeit : 6
Rührgeschwindigkeit : 6
Ultraschall : 02:00 (7)
Art der Verteilung : Volumen

D10 : 1.54701(µm) 
Median : 4.73807(µm) 
D90 : 18.55172(µm)
Laser Scattering Particle Size Distribution Analyzer LA-950

ID# : 201208080948840
Probenname : 13922_Horse teeth bone_CryoMill_25mlss_20mmball_8x2min
Datenbezeichnung : 13922_Horse teeth bone_CryoMill_25mlss_20mmball_8x2min
Quelle :
Transmission (R) : 87.3(\%) %
Transmission (B) : 82.0(\%) %
Zirkulationsgeschwindigkeit : 6
Rührgeschwindigkeit : 02:00 (7)
Art der Verteilung : Volumen

D10  :  1.20147(\µm)
Median  :  2.57494(\µm)
D90  :  7.26641(\µm)

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Laser Scattering Particle Size Distribution Analyzer LA-950

ID# : 201208081153861
Probenname : 13922_Horse Mandibula_CryoMill_25mlss_20mmball_8x2min
Datenbezeichnung : 13922_Horse Mandibula_CryoMill_25mlss_20mmball_8x2min
Quelle :
Transmission (R) : 85.6(\%)
Transmission (B) : 79.7(\%)
Zirkulationsgeschwindigkeit : 6
Rührgeschwindigkeit : 6
Ultraschall : 02:00 (7)
Art der Verteilung : Volumen

D10 : 1.53918(\µm)
Median : 4.20583(\µm)
D90 : 10.37547(\µm)