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Evaluation of sample preparation (grinding and sieving) of bivalves, coffee and cowpea beans for multi-element analysis

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Abstract

Elemental analyses of food samples require several pre-treatment steps that constitute a great potential source of errors. In this study, the influence of cryogenic, ball and knife mill devices and also sieving using different sizes of sieve (100, 300 and 500 μm) was evaluated for samples of bivalves, coffee and cowpea beans. A two-factor ANOVA was performed in each sample to test for differences between macro, micro and trace element concentrations determined by ICP OES. Results showed that the efficiency of the particle size reduction and sample homogeneity depends on the milling device and the nature of samples. Food samples may present segregation after comminution, and sieving might become a necessary step. Nevertheless, the sieve aperture has to be chosen cautiously, once it might influence the final element concentration. Overall, the expected results by employing cryogenic grinding, such as rapid sample homogenization and small particle size generated were also observed for ball mill. Contamination can be a critical issue for some elements and need to be evaluated individually.

Keywords: Sample preparation; Grinding; Sieving; Food samples; Multi-element analysis

1. Introduction

Comminution represents one of the most important steps in sample treatment, and one of those that most likely contribute to erroneous results. Comminution refers to the grinding of large particles into a powder. Generally, the comminution is based upon the collision between sample and mill surface, which might result in sample contamination [1]. Small particles have great surface areas, are more homogenous and are more easily decomposed [2], improving the procedures of dissolution and extraction. The particle size as well as the size distribution can affect significantly the composition of a sample in terms of homogeneity and representativity of sub-samples, which can be easily observed in techniques that use small masses of samples (e.g. <100 mg) [3]. That is the case in modern instrumental analysis (e.g. solid sampling atomic absorption spectrometry, particle-induced X-ray emission, and laser plasma spectrometry), hence sample homogeneity is essential to obtain representative sub-samples.

There is a number of different grinding equipment available commercially. The models can differ in the technique used for grinding (e.g. cutting, pressure, friction, and impact of two surfaces), the material (e.g. tungsten carbide, agate, or steel), capacity and particle size distribution obtained [4]. It is possible to distinguish comminution efficiency regarding the particle size range generated, i.e. coarse size >5 mm, fine size >63 μm and extra-fine size <63 μm. The duration of the comminution process and the number of cycles to which samples are submitted are important factors for the efficient reduction of particle sizes [5]. Nevertheless, the nature, complexity and characteristics of a sample are also important in the determination of the resulting

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size. Some types of beans, for instance, are not easily ground in a variety of grinding mills. In this case, sieving of ground samples is usually necessary to separate the outer shell and inner parts of beans. The grinding process can also be unsatisfactory in food samples containing high contents of oils or fats, such as Brazilian nuts [6] or fish samples [7].

Especially for trace element analysis, the comminution, in any kind of mechanical grinding equipment and sieving procedures might contaminate the samples being ground [2]. The element to be analyzed should not be present in the material of the grinding equipment and sieve used. Another important issue associated with some grinding equipments is the loss of volatile elements due to the overheating generated during the comminution.

The aim of this study was to evaluate the use of three milling devices (cryogenic, ball and knife) for the homogenization of coffee, cowpea beans and bivalves for the determination of major, minor and trace elements by inductively coupled plasma optical emission spectrometry. The influence of sieving, using different sizes of sieve (100, 300 and 500 μm) was also evaluated. The results are discussed in terms of the potential of contamination and fractioning of samples, loss of volatile elements and particle size generated.

2. Experimental

2.1. Instrumentation

Three milling devices were tested. A cryogenic mill model MA 775 (Marconi, Brazil), with a polycarbonate grinding vial supplied with two stainless steel plugs, immersed in liquid nitrogen and ground with an alternating magnetically driven steel impactor. A conventional stainless steel knife micro-mill, model MA 630 (Marconi, Brazil) and a ball mill, model 8000 M (Spex Sample Prep, USA) with a tungsten carbide vial set and tungsten carbide ball. A closed-vessel microwave digestion system (ETHOS EZ, Milestone, Italy) was used for sample decomposition.

An inductively coupled plasma optical emission spectrometer (ICP OES) with axially viewed configuration (VISTA PRO, Varian, Mulgrave, Australia) equipped with solid state detector, cyclonic spray chamber, and concentric nebulizer was employed for Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, P, Sr, Sb, Se and Zn determinations. The operating conditions are summarized in Table 1.

Table 1
Instrumental parameters for multi-element ICP OES determinations

Optical system	Echelle
Detector	Solid CCD (167–785 nm)
Power (W)	1300
Plasma gas flow rate (L min^{-1})	15
Auxiliary gas flow rate (L min^{-1})	1.5
Nebulizer argon gas flow rate (L min^{-1})	0.70
Sample flow rate (L min^{-1})	0.70
Replicate read time (s)	1
Instrument stabilization delay (s)	15
Pump rate (rpm)	15
Spectral lines (nm)	Al I 396.152; As I 188.980; Ba II 455.395; Ca II 396.847; Cd II 226.502; Co II 230.786; Cr II 267.716; Cu I 327.398; Fe II 259.940; K I 766.465; Mg II 279.551; Mn II 257.610; Ni II 231.302; P I 213.618; Pb II 220.352; Sr II 407.771; Sb I 206.834; Se I 196.026; Zn I 213.857

I: atomic line; II: iosnic line.

2.2. Reagents, solutions, reference materials and samples

Nitric acid, 65% w/w, and hydrogen peroxide, 30% v/v (Merck, Darmstadt, Germany) were used for sample decomposition. Ultrapure water (Milli-Q®, Millipore, USA) with resistivity lower than 18.2 MΩ cm was used throughout. The multi-element reference solutions were prepared daily from 1000 mg L^{-1} stock solutions of each element (Titrisol®, Merck, Germany). A working standard solution containing 50 mg L^{-1} Al, Cu, Fe, Mn, Zn and 10 mg L^{-1} As, Ba, Cd, Co, Cr, Ni, Pb, Sr, Sb and Se was used. For the major elements a working solution was prepared containing 50 mg L^{-1} Ca, Mg, P and 100 mg L^{-1} K. Analytical curves were prepared using the standard calibration technique.

Three different homogenized food samples were employed for evaluating grinding and sieving procedure performance: roasted coffee beans (*Coffea arabica*), dry and mature cowpea beans (*Vigna unguiculata L.*) and bivalves (*Anomalocardia brasiliana*).

The certified reference material Oyster Tissue (NIST SRM 1566b; National Institute of Standards and Technology, Gaithersburg, MD, USA) was used to check the accuracy of sample decomposition and element determinations. There are no certified reference materials available for coffee and cowpea beans.

2.3. Sample preparation

Bivalve samples were freeze dried, and maintained in a dessiccator at room temperature. Coffee and cowpea beans were kept under refrigeration.

For the grinding of samples the first aliquot was discarded. A two-step program was used for cryogenic grinding. Samples were frozen for 5 min and ground for 2 min. Three grinding cycles with a cooling step of 1 min between cycles were applied. Three cycles of 2 min were used for grinding samples in the ball and knife mills. Aliquots of ground samples were sieved in nylon sieves of 100, 300 and 500 μm.

After grinding and sieving, approximately 250 mg of each sample was digested, in the microwave oven, using 7.0 mL of HNO_3 65% w/w and 1.0 mL of H_2O_2 30% v/v in closed PFM vessels. The heating program was performed in four successive steps. In the first step the temperature was linearly increased to 90° C in 4 min with maximum power of the rotating magnetron of 1000 W. In the second step, the temperature was kept at 90 °C

Table 2
Ranges of elemental concentration for bivalves, coffee and cowpea beans after milling (cryogenic, ball and knife) and sieving (100, 300 and 500 μm mesh)

Elements	Concentration range (μg g^{-1})		
	Cowpea beans	Coffee beans	Bivalves
Al	3.10–8.13	15.6–26.2	652–785
As	<13	<13	4–13
Ba	4.44–12.2	0.754–1.04	3.37–3.80
Cd	<0.1	<0.1	1.56–1.79
Co	<0.8	<0.8–1.1	3.45–5.30
Cr	<0.7	<0.7	1.70–5.09
Cu	3.74–4.47	13.4–14.2	9.64–11.1
Fe	44.3–54.2	46.9–94.4	531–682
Mn	18.4–25.1	17.8–19.3	33.1–58.6
Mo	1.31–1.77	<1.1	2.05–2.52
Ni	1.48–2.04	<0.8	5.60–6.15
Pb	<2.6–2.79	<2.6	<2.6
Sb	<12	<12	<12
Se	<5.8	<5.8	<5.8
Sr	3.99–8.17	4.51–5.50	59.5–63.9
V	<0.6	<0.6	0.75–1.0
Zn	39.1–43.5	5.27–6.28	68.0–76.8
Ca	342–704	1227–1437	5684–6475
K	11,525–12,665	19,435–20,111	10,882–11,296
Mg	1226–1763	2145–2246	4981–5182
P	3379–4884	2660–3067	16,580–17,353

for 2 min. In the third step, the temperature was linearly increased to 180° C in 8 min and, in the fourth step the temperature was kept at 180° C for 15 min. After digestion and cooling, which took about 2 h, the solutions were transferred to plastic flasks and made up to a final volume of 25.0 mL with water. Three replicates were prepared for each sample. Blanks were prepared in the same way as samples and were analyzed in each batch of samples.

2.4. Experimental design and data analysis

A two-factor analysis of variance (ANOVA) was performed on each sample type (i.e. bivalves, coffee and cowpea beans) to test differences between element concentrations in samples ground in cryogenic, balls and knife mills, and sieved in 100, 300 and 500 μm sieve. Factors in these analyses were "Mill" (fixed; three levels) and "Sieve" (fixed and orthogonal; three levels). Cochran's test was used to test the homogeneity of variances. If variances were heterogeneous, the data was transformed [8]. For some elements data transformation did not remove heterogeneity, hence untransformed data was used. Interpretations of results for elements, the variances of which were heterogeneous were made cautiously, noting the robustness of ANOVA, where balanced designs and sufficient replicates were used [8]. Multiple comparisons among the means were performed using Student–Newman–Keuls (SNK) tests. These analyses were done using the program GMAV-5.

3. Results and discussion

Initially, experiments were performed to characterize the composition of each sample (i.e. bivalve, coffee and cowpea beans). The concentration ranges of the studied elements for bivalve, coffee and cowpea beans, after samples being ground and sieved, are presented in Table 2. It is clear that samples, based on the studied elements, had a different composition. In general, concentrations of trace and major elements were higher in bivalves compared to coffee and cowpea beans. However, the concentration of K was higher in coffee samples than in cowpea beans and bivalves. Comparing bean samples, concentrations of Zn were much higher in cowpea than in coffee, whereas concentrations of Ca, K, Mg, Al and Cu were higher in coffee than in cowpea beans.

The three studied matrices were not only different in composition but also in texture, hardness, and fat content, which made these food samples good matrices to test artifacts regarding sieving and grinding procedures.

The accuracy of the procedures was evaluated using the certified material Oyster Tissue (NIST 1566b — National Institute of Standard and Technology). The results, showed in Table 3, confirm the accuracy and precision of the analytical procedures employed. Concentration of Al, nevertheless, indicated low recoveries when compared to certified values.

3.1. Comminution and sieving effects: ANOVA results

Samples of coffee beans, cowpea beans and bivalves presented significant variation in major and minor element concentrations after sieving and grinding in cryogenic, ball and knife mills (Table 4, 5, and 6, respectively). However, the pattern of variation differed between studied matrices and elements.

Coffee beans were the matrix that presented the smallest number of significant interactions between sieve and milling devices (Table 4). Copper, K and Mg concentrations were not altered after being milled and sieved, regardless of the procedure used. The sieving procedure was significant only for Ba and Ca, whereas Al, Mn, Sr and Zn presented significant interactions between sieves and milling devices. For most of the cited elements the smallest concentration was observed in samples sieved through 100 μm sieve. Comminution in

Table 3
Results ($n=4$) of certified reference material (Oyster tissue, NIST SRM 1566b)

Element	Certified values (μg g^{-1})	Obtained values (μg g^{-1})
Ca*	0.0838±0.0020	0.0826±0.0037
K*	0.652±0.009	0.562±0.008
Mg*	0.1085±0.0023	0.1028±0.0033
Al	197.2±6.0	96.3±6.1
As	7.65±0.65	7.30±1.09
Ba	8.6±0.3	7.85±0.18
Cd	2.48±0.08	2.43±0.07
Co	0.371±0.009	0.351±0.008
Cu	71.6±1.6	69.7±1.7
Fe	205.8±6.8	205.3±5.5
Mn	18.5±0.2	18.2±0.6
Ni	1.04±0.09	1.10±0.11
Sr	6.8±0.2	6.6±0.2
V	0.577±0.023	0.548±0.052
Zn	1 424±46	1 369±24

*%.

Table 4
Summary of two-factor ANOVA of major and trace element concentrations in coffee bean samples ground in ball, cryogenic and knife mills and sieved through 500, 300 and 100 μm mesh

Coffee beans				
	Mill	Sieve	Mill × Sieve	
			Mi (Si)	Si (Mi)
Al*	–	–	C > K ≈ B (5)	ns
Ba*	B ≈ K > C	5 ≈ 3 > 1	ns	ns
Ca	K > C > B	3 > 5 > 1	ns	ns
Co *t*	B > K ≈ C	ns	ns	ns
Cu	ns	ns	ns	ns
Fe*	C > K ≈ B	ns	ns	ns
K	ns	ns	ns	ns
Mg	ns	ns	ns	ns
Mn	–	–	C > B > K (5)	ns
P	C > B > K	ns	ns	ns
Sr	–	–	K > C ≈ B (3) (1)	3 > 5 > 1 (K) 5 ≈ 3 > 1 (C)
Zn	–	–	C ≈ K > B (1)	1 > 5 ≈ 3 (K) (C)

ns = not significant; – = interaction Mill × Sieve with $P<0.05$; Mi = Mill (K = Knife, C = Cryogenic, B = Ball); Si = Sieve (5 = 500 μm, 3 = 300 μm, 1 = 100 μm); * = heterogeneous variance; *t* = data was log ($x+1$) transformed.

cryogenic mill resulted in the highest concentrations of Fe and P, and also Al and Mn, for samples sieved in 500 μm and Zn for samples sieved in 100 μm. Barium and Ca concentrations were enriched after being ground in the knife mill. Amorin Filho et al.

Table 5
Summary of two-factor ANOVA of major and trace element concentrations in cowpea bean samples ground in ball, cryogenic and knife mills and sieved through 500, 300 and 100 μm mesh

Cowpea beans				
	Mill	Sieve	Mill × Sieve	
			Mi (Si)	Si (Mi)
Al *t*	ns	ns	ns	ns
Ba	–	–	C > K > B (3) (1)	5 > 3 > 1 (K) (B) 5 ≈ 3 > 1 (C)
Ca	–	–	C > K > B (3) C > F ≈ B (1)	5 > 3 > 1 (K) (B) 5 ≈ 3 > 1 (C)
Co*	–	–	B > K ≈ C (5) (1)	5 ≈ 1 > 3 (B)
Cu	–	–	K > C ≈ B (3) (1)	5 > 3 > 1 (B)
Fe	–	–	C > K ≈ B (1)	5 ≈ 3 > 1 (K) 5 > 3 ≈ 1 (B)
K	–	–	K > C ≈ B (1)	3 > 5 > 1 (B)
Mg	–	–	K > C ≈ B (5) C > K > B (3) (1)	5 > 3 > 1 (K) 5 ≈ 3 > 1 (C) (B)
Mn*	–	–	B > K ≈ C (5) C > K ≈ B (1)	5 ≈ 3 > 1 (K) 5 > 3 > 1 (B)
Mo*	ns	5 > 3 ≈ 1	ns	ns
Ni	ns	5 ≈ 3 > 1	ns	ns
P*	–	–	C ≈ B > K (5) K ≈ B > C (1)	5 > 3 ≈ 1 (C)
Sr *t*	–	–	C > K > B (3) (1)	5 > 3 > 1 (K) (B) 5 ≈ 3 > 1 (C)
Zn	–	–	C ≈ K > B (1)	5 ≈ 3 > 1 (B)

ns = not significant; – = interaction Mill × Sieve with $P<0.05$; Mi = Mill (K = Knife, C = Cryogenic, B = Ball); Si = Sieve (5 = 500 μm, 3 = 300 μm, 1 = 100 μm); * = heterogeneous variance; *t* = data was log ($x+1$) transformed.

Table 6
Summary of two-factor ANOVA of major and trace element concentrations in bivalve samples ground in ball, cryogenic and knife mills and sieved through 500, 300 and 100 μm mesh

Bivalves				
	Mill	Sieve	Mill × Sieve	
			Mi (Si)	Si (Mi)
Al	+	ns	ns	ns
As	ns	ns	ns	ns
Ba*	ns	ns	ns	ns
Ca	–	–	B > K ≈ C (3)	5 ≈ 3 > 1 (B)
Cd	–	–	C ≈ K > B (1)	1 > 5 ≈ 3 (K) (C)
Co	B > C > K	1 > 5 ≈ 3	ns	ns
Cr	–	–	K > C > B (5) (3) (1)	1 > 5 ≈ 3 (K)
Cu	–	–	C > K ≈ B (5) C > K > B (3) C ≈ K > B (1)	1 > 5 ≈ 3 (K) (C)
Fe	–	–	K > B ≈ C (5) K > B > C (1)	1 > 5 ≈ 3 (K) 5 ≈ 3 > 1 (C)
K	ns	ns	ns	ns
Mg	ns	ns	ns	ns
Mn	–	–	K > C > B (5) (3) (1)	1 > 5 ≈ 3 (K)
Mo	ns	ns	ns	ns
Ni	ns	ns	ns	ns
P	K > C ≈ B	ns	ns	ns
Sr	–	–	C ≈ B > K (3) C ≈ K > B (1)	1 > 5 ≈ 3 (K) 3 > 5 > 1 (B)
V	ns	ns	ns	ns
Zn	K > C ≈ B	ns	ns	ns

ns = not significant; – = interaction Mill × Sieve with $P<0.05$; Mi = Mill (K = Knife, C = Cryogenic, B = Ball); Si = Sieve (5 = 500 μm, 3 = 300 μm, 1 = 100 μm); * = heterogeneous variance; + = $P<0.05$ but a posteriori test (SNK) failed to detect differences.

[9] have shown that a knife mill (Willye-type) contaminated coffee samples with Fe, when compared to cryogenic mill. It is important to note that the composition of the materials from which the mill devices are made can be quite different, hence even the same type of mill can result in different kind of contamination depending on its material (e.g. steel, titanium, tungsten carbide) and also the hardness and composition of the samples. Cobalt and Ba concentrations were significantly altered by the ball mill.

The analysis of cowpea beans showed that almost all elements presented significant interactions between the milling device and the sieve, Al, Mo and Ni being the only exceptions (Table 5). Segregation of sample seems to be a critical issue for cowpea beans. For instance, sieving in 100 μm and 500 μm sieve resulted in the lowest and highest concentrations, respectively, of most of the studied elements when compared to aliquots sieved in 300 μm sieve (Table 5, Si (Mi) interaction). The artifact effects of the milling devices occurred for most studied elements, mainly in samples sieved in 100 μm (Table 5, Mi (Si) interaction). The concentrations of Fe and Mn for samples sieved in 100 μm, and concentrations of Ba, Ca, Mg and Sr for samples sieved in 100 and 300 μm were significantly higher in samples ground in the cryogenic mill when compared to aliquots ground in other devices. Samples ground in the knife mill presented the highest concentration of Cu (100 and 300 μm sieves), Mg (500 μm) and K (100 μm). The ball mill had a

limited influence in cowpea beans, altering only the concentrations of Mn (500 μm sieve) and Co (100 and 500 μm sieves).

For bivalves various elements (i.e. As, Ba, K, Mg, Mo, Ni, and V) did not show significant artifact effects due to comminution and/or sieving processes (Table 6). For most of the elements that presented a significant milling device influence or an interaction between mill type and sieve used (Table 6, Mi (Si) interaction), the lowest concentration of elements occurred when ball mill was employed. In fact, the ball mill appeared to have the smallest effects on element concentrations for the three samples studied. The only exception was Co, which presented the highest concentration in samples of bivalves, coffee and cowpea beans after the comminution in the ball mill. This is not unexpected, once the ball mill used in this study was made of tungsten carbide, which contains approximately 6% of Co [2]. Moreover, the samples that were ground in the ball mill were not subject to relatively high temperature, which could cause losses due to volatilization. Samples were ground in 2 min cycles, which was not enough to reach temperatures above 40 °C. Cryogenic and knife mills had the same effect on Cd, Cu and Sr in aliquots sieved in 100 μm sieve (Table 6, Mi (Si) interactions). Nevertheless, samples ground in the cryogenic mill resulted in the highest concentration of Cu (300 and 500 μm sieves), whereas concentrations of Cr, Mn, and Fe (100 and 500 μm sieves), were the most altered by knife milling. The sieving procedure changed significantly the results of less than 40% of the studied elements. There was no clear pattern in the sieve influence on bivalve samples. In fact, bivalves are easily ground in any of the studied mills, hence the sieving after grounding is an unnecessary step.

The NIST SRM Oyster Tissue is a homogenized powder, ready to be dried and digested. Nevertheless, aliquots of this sample were also processed in the three studied milling devices, but they were not sieved. Comparing the results of the aliquots analyzed without comminution and the ones ground in ball, knife and cryogenic mills, a contamination with Cr and Fe could be observed when cryogenic mill was used. The contamination of samples with Cr, Fe and also Ni is well documented for cryogenic grinding [7], whereas more recent work reported no contamination with Fe [6,10,11]. The difference between the contamination effects of the oyster tissue reference material (Table 3) and the bivalves (Table 2) analyzed here can, at least in part, be due to the composition of these matrices. For instance concentrations of Al, Cr, Fe, Ni and V were much higher in bivalves than in oyster tissue, which makes it more difficult to detect contamination effects.

3.2. Sample homogeneity

The homogeneity of bivalves, cowpea and coffee bean samples was evaluated considering the mean relative standard deviation (RSD%) for Ca, Cu, P, K, Mg, Mn, Sr and Zn obtained using the three tested grinding procedure for each matrix (Fig. 1). These elements were chosen based on the concentration range in which they occurred in each matrix. There was not much discrepancy between the values of these

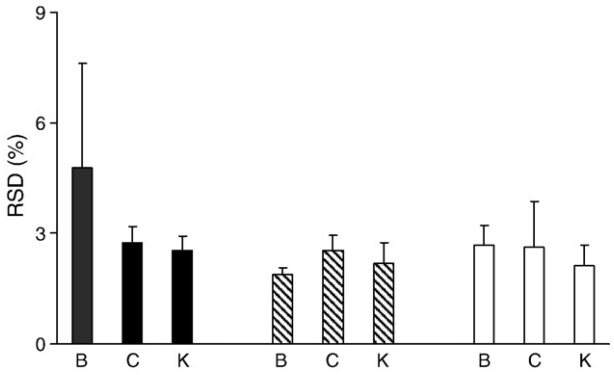

Fig. 1. Mean relative standard deviation and standard deviation values for Ca, Cu, K, Mg, Mn, P, Sr, and Zn for samples of cowpea beans (full), coffee beans (dashed) and bivalves (open) ground with ball (B), cryogenic (C), knife (K) milling devices.

elements in the three studied samples, hence, it is expected that the error associated with element determinations in each matrix was similar.

Comparing the three studied food samples, it is clear that the cowpea beans were the matrix that presented the highest variability (Figs. 1 and 2). This is certainly the result of the morphology of the beans, which consists of a hard shell (tegument) covering the inner part (endosperm and embryo) of the bean. The hardness and composition of these parts are different and if the comminution process is not efficient it might result in heterogeneity and segregation of the sample. The heterogeneity of the cowpea bean samples was most evident in aliquots ground in the knife mill, as it can be seen in Fig. 2.

The bivalves and coffee beans presented similar RSD values for cryogenic and knife mills. The performance of the ball mill was slightly better for coffee beans than for bivalves. Moreover, the difference in the RSD values among tested milling devices for coffee beans were also the smallest, suggesting that this matrix is more easily comminuted, and that the milling devices tested would have similar effects on sample homogeneity. It is important to note that for this study roasted coffee beans were used. The commercialized roasted coffee undergoes a series of processes. For instance, raw coffee beans are dehydrated, the shell is eliminated and the beans are roasted (≈220 °C). These pre-treatments have an important effect increasing the brittleness of the beans and also reducing sample heterogeneity.

3.3. Particle size and morphology

The grinding procedure converts the solid sample into a powder, drastically changing particle size and morphology. It can be seen from the micrographs (Figs. 2–4) that the studied food samples have distinct characteristics due to their structural differences and the preparation process (i.e. grinding and sieving). In general, samples comminuted with the knife mill presented the largest range of particle sizes, being the least efficient comminution process (Table 7).

The cowpea beans (Fig. 2) showed regular oval shape which is compatible with the starch format of the mature bean [12]. A substantial amount of fragments attached to the surface of starch

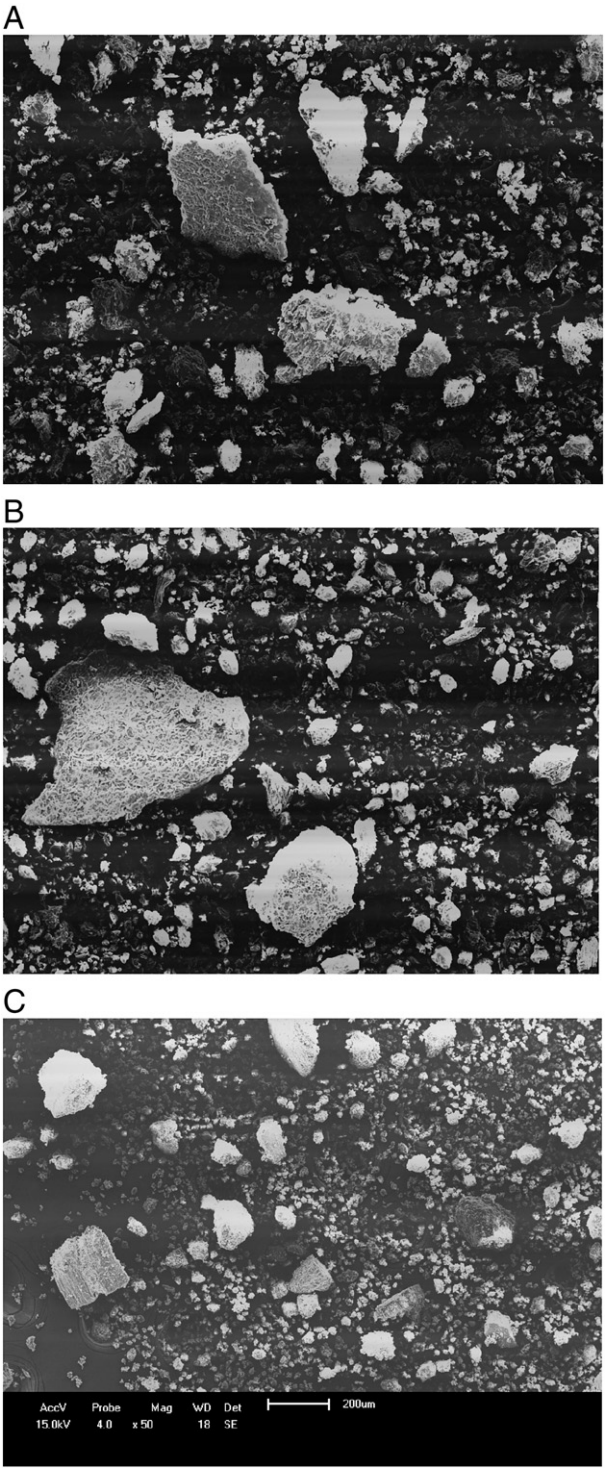

Fig. 2. Scaning electron microscopy of particles generated for cowpea bean samples after grinding and sieving in 500 μm sieves. (A) ball mill; (B) knife mill; (C) cryogenic mill.

granules are, probably, from cell walls, which are rich in protein and keep the starch cell blinded together. It was observed that the cryogenic mill produced very fine structures that tended to aggregate to each other. These fine structures, particularly in cowpea beans, adhered to the starch surface. The cowpea starch granules, obtained by the knife mill, presented a small amount of fragments adhered to surfaces. Moreover, particles showed a rounded shape, similar to the ones obtained from the ball mill. With regard to the sieve sizes used (Table 7), particularly for cowpea beans, large structures were observed in the 500 μm sieve (Fig. 2), which could be due to the great difference in the plasticity of the components of the beans (i.e. tegument, endosperm and embryo).

It was not possible to observe structural differences in coffee samples milled in the different devices (Fig. 3). The severe

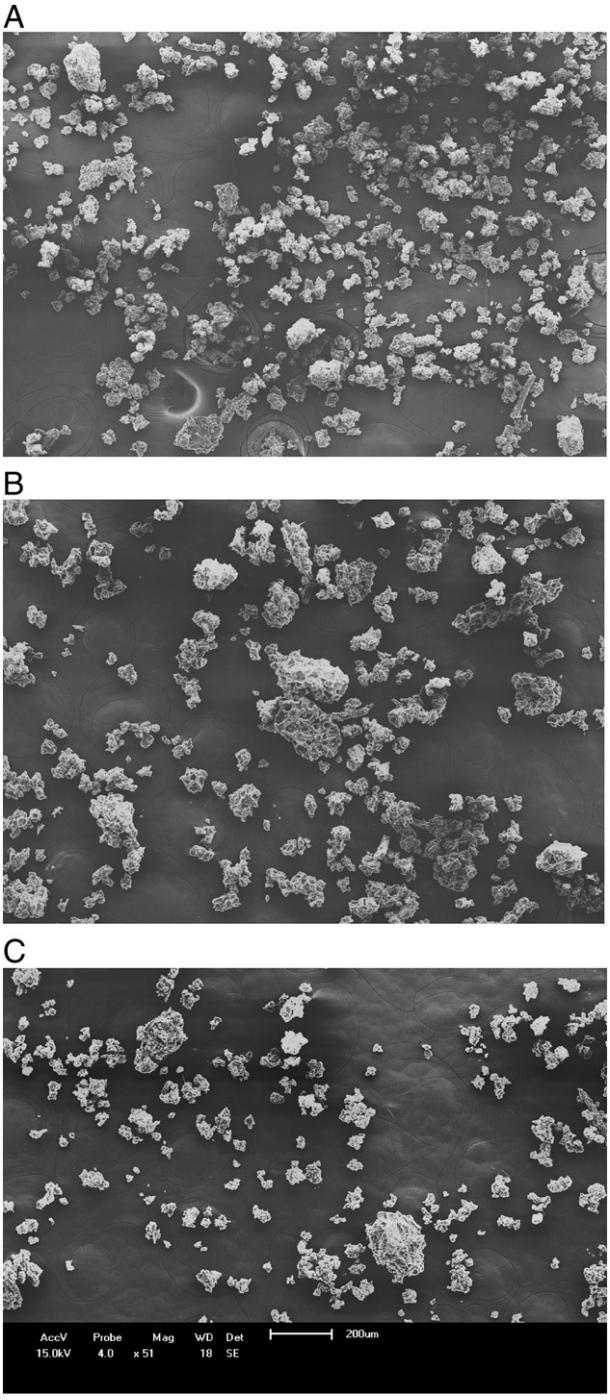

Fig. 3. Scaning electron microscopy of particles generated for coffee samples after grinding and sieving in 500 μm sieves. (A) ball mill; (B) knife mill; (C) cryogenic mill.

thermal action of the roasting process seems to affect the coffee cell structures that lose their integrity and functionality, particularly the structural proteins and the fibers responsible for maintaining the cell walls. Therefore, the comminution of coffee beans, regardless the milling device employed, is facilitated. At least in part, the roasting process, could also explain why sieving have a limited influence on major and minor element concentrations (Table 4) compared to the other matrices.

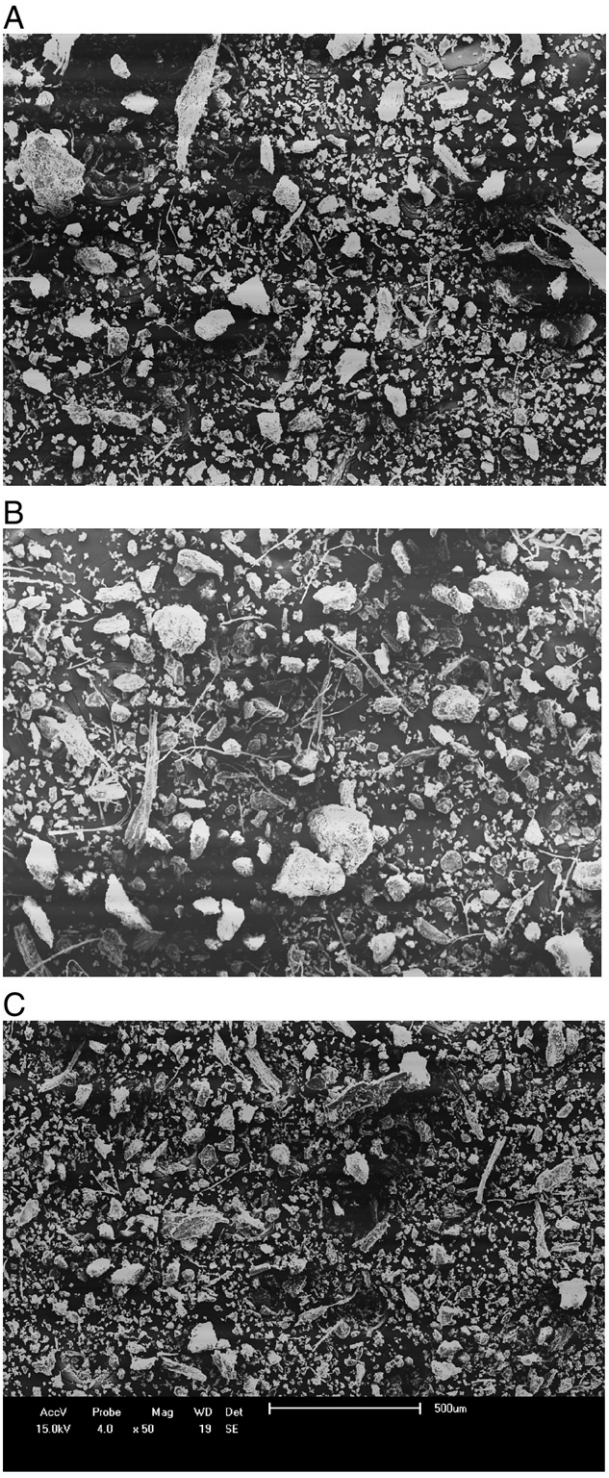

Fig. 4. Scaning electron microscopy of particles generated for bivalve samples after grinding and sieving in 500 µm sieve. (A) ball mill; (B) knife mill; (C) cryogenic mill.

Table 7
Particle size range of bivalves, coffee and cowpea bean samples after being milled and sieved

Mill	Sieve (µm mesh)	Particle size range (µm)		
		Coffee beans	Cowpea beans	Bivalves
Ball	100	20–100	20–100	10–160
	300	20–100	50–290	10–380
	500	30–190	20–270	10–430
Knife	100	50–100	15–90	30–700
	300	50–250	30–300	10–700
	500	30–370	20–500	20–700
Cryogenic	100	10–90	20–90	10–120
	300	20–100	15–210	40–390
	500	15–260	30–230	10–530

Bivalves comminuted in all tested devices presented polyedric structures of sharp angles. The polyedric structures were more abundant when the sample was ground in the knife mill, whereas a more rounded shape was observed when they were comminuted in the ball mill (Fig. 4). Moreover, bivalve samples comminuted in the knife mill showed elongated structures with length varying between 400 to 700 µm (Fig. 4), which can be associated with the presence of insoluble fibers of high plasticity. These structures occurred in major quantity in the sieves of 300 and 500 µm, while they were less abundant in samples comminuted in the cryogenic mill. The latter is possibly associated with the brittle fracture at low temperatures (approximately −190 °C).

4. Conclusions

Cryogenic, ball and knife mills can be used to reduce particle size and obtain homogeneity in food samples. The performance of each mill varies with the texture and hardness of the samples, and sieving might be a necessary step to suppress the effect of segregation in samples such as cowpea beans. Moreover, the aperture of the sieve used may influence significantly the final concentration of elements. The choice of the type of milling device should take into account the availability of mills, sample type and elements to be determined, once the occurrence of contamination can be a critical issue. Overall, the ball mill presented a good performance for all sample types and it is an interesting alternative to the cryogenic mill, once maintenance costs are much lower. Nevertheless, the contamination of Co by the ball mill was detected in all three studied food samples.

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