

ED-XRF as a Tool for Rapid Minerals Control in Milk-Based Products

LOÏC PERRING* AND DANIEL ANDREY

Minerals Laboratory, Department of Quality and Safety Assurance, Nestlé Research Centre,
 Vers chez-les-Blanc, CH-1000, Lausanne26, Switzerland

An ED-XRF method for the rapid determination of a series of analytes (phosphorus, sulfur, chlorine, potassium, calcium, iron, zinc) in milk-based products has been developed and validated. The investigated samples were commercial products obtained from various parts of the world. Reference values measured by inductively-coupled plasma-optical emission spectroscopy and by potentiometry for chloride were used to calibrate the ED-XRF. Calibrations were established with 30 samples, and validation was made using a second set of 30 samples. An evaluation of this alternative method was done by comparison with data from the reference methods. Pellets of 4 g were prepared under 2 tons of pressure. For each sample, 3 pellets were prepared and analyzed. Limits of quantification and repeatabilities were evaluated for the described analytes.

INTRODUCTION

A great advantage of X-ray fluorescence technique compared to wet chemical analysis is that the measurement can be directly carried out on solid samples. This avoids sample digestion-dissolution, using toxic and corrosive acids. Less preparation and manipulation means time and cost savings. Furthermore, the use of XRF for chlorine is interesting, because it cannot be determined by classical atomic absorption or inductively coupled plasma (nonequipped with deep-UV optics: 120–180 nm). These reference methods require qualified personnel, for daily instrument calibrations using standard solutions and preparation of samples prior to analysis. This sample preparation step is time-consuming, and contamination may occur (e.g., iron) from the laboratory environment, leading to inaccurate results. Application of good laboratory practices allied to a suitable internal control plan are essential to obtain accurate results.

For two decades, solid foodstuff materials have been analyzed by ED-XRF. As foods (starting materials or finished products) are being more and more surveyed, it is a major challenge to provide rapid, multi-element and accurate techniques to obtain control data about process, toxicity, or nutrition/health. Radioactive sources have been used in the past for excitation (1–4) or X-ray tubes (5–10), most of the authors using semiconductor detectors. Various foodstuff types were covered, such as bread improver (1), spices (2), tea (3–5), flours (4–8), milk powders (6–7), vegetables-fruits-grains (8–9), and fast-food samples (10). These studies described laboratory quantification of analytes of interest, with elemental fingerprinting for qualitative information about origins or processes. None of the papers mentioned the potential of ED-XRF for quality control in production sites; some even exist in suppliers application notes.

Thus, the XRF technique shows potential as an analytical tool to be used near the food production lines because direct analyses of solid samples can be performed without any pretreatment. In the dairy field, to ensure correct addition of mineral premixes during infant formula production, tracers (e.g., iron) may be determined in final products. Accurate and rapid analysis of the iron tracer can be used to modify the process parameters and thus to ensure target concentration of premixes added to the milk powders is achieved. When the target concentration is obtained, the infant formula production can be released. For this kind of control, the use of the energy-dispersive X-ray fluorescence technique shows potential.

In the present study, a suitable range of products of milk-based matrixes, containing a wide range of mineral values, was used to establish calibrations. The ED-XRF intensity values, as well as the reference elemental values are stored in the PC controlling the apparatus. The aim of this work was to evaluate the analysis of several elements: phosphorus, sulfur, chlorine, potassium, calcium, iron, and zinc in milk-based products by Energy Dispersive-X-ray Fluorescence.

MATERIAL AND METHODS

Samples. All samples were commercial milk-based powders. Two groups of samples were used during this study.

Calibration set: a series of commercial worldwide products (30 samples).

Validation set: a series of similar commercial worldwide samples (30 samples).

Apparatus. ED-XRF Xepos from Spectro (Kleve-Germany) was used for this study. All ED-XRF measurements were made on three different pellets of the same product. Pellets were pressed using a manual hydraulic press (max pressure, 5 tons) (Specac, Kent, United Kingdom). The diameter of the pellet die (Specac was 32 mm).

References values of the all samples were measured using in-house validated procedures by ICP–OES for P, S, K, Ca, Fe, and Zn and by potentiometry for Cl. Analyses were systematically made in duplicate.

* Author to whom correspondence should be addressed. E-mail: loic.perring@rdls.nestle.com.

Table 1. Origin of the Powders

origin	no. of samples
Brazil	3
Danmark	6
France	8
Germany	8
Guatemala	1
India	3
Malaysia	5
Netherland	7
Russia	2
Spain	4
Switzerland	7
(local market)	
Thailand	6

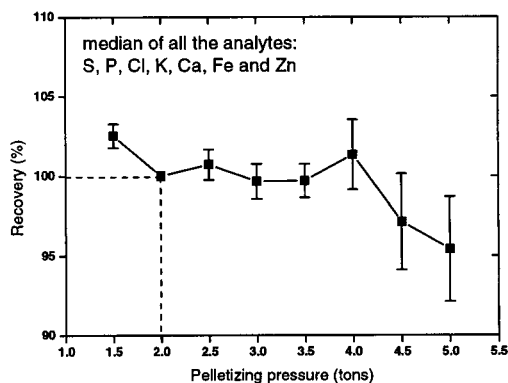


Figure 1. Influence of pressure applied to the pellets on recovery of analytes (2 tons considered as 100% of accuracy).

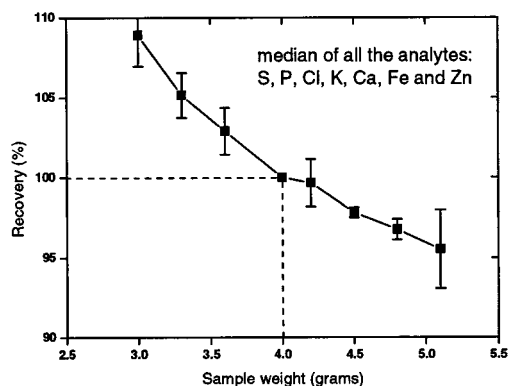


Figure 2. Influence of pellet weight on recovery of analytes (4 g considered as 100% of accuracy).

A Varian Vista-AX Axial ICP-OES (Varian, Australia) equipped with a charge coupled device (CCD) detector was used. A cyclonic spray chamber (thermostated at 15 °C), coupled with a Micromist nebulizer (Glass Expansion, Australia), was used as injection device, giving a signal of higher precision. Chloride quantifications were done using a titration unit: Titrino DMS 716 + sample changer 730 + electrode DM 141-SC (Metrohm, Switzerland) and following the silver nitrate titration method.

Preparation of Samples for ED-XRF Analysis. It was found that a constant pressure and weight have to be applied to only take into account analytes concentrations and matrix effect (11) as factors of variability of XRF intensities. Several parameters were modified to evaluate their effects on the ED-XRF results: pellet weight, and pelletizing pressure.

Figures 1 and 2 show that modifications of pelletizing pressure or powder weight lead to a significant difference of concentrations for all the studied analytes, due to density variations of pellets and quantity of powder involved in XRF process fluorescence radiations. Final parameters were then fixed as follows: 4 g of powder at 2 tons. Fat

Table 2. Measurement Conditions

	condition 1	condition 2
atmosphere	vacuum	vacuum
X-ray tube target	palladium compton secondary molybdenum	palladium HOPG
voltage, current	45 kV, 0.55 mA	25 kV, 1.00 mA
acquisition time	300 s	
detector	Xflash-LT with a Be entrance window	
number of pellets	3	
weight of pellet	4 g	
pellet diameter	32 mm	
pelletizing pressure	2 tons	
analytes	Fe, Zn	P, S, Cl, K, Ca

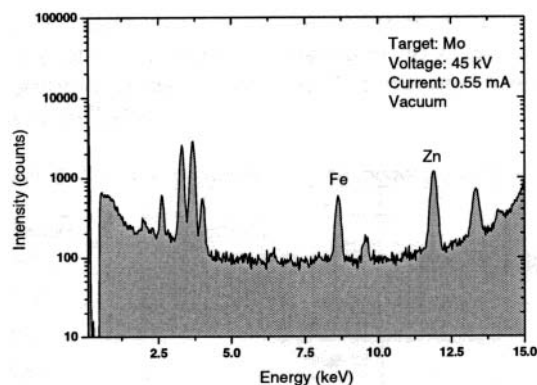


Figure 3. Condition 1: Example of spectrum obtained for Fe and Zn measurement.

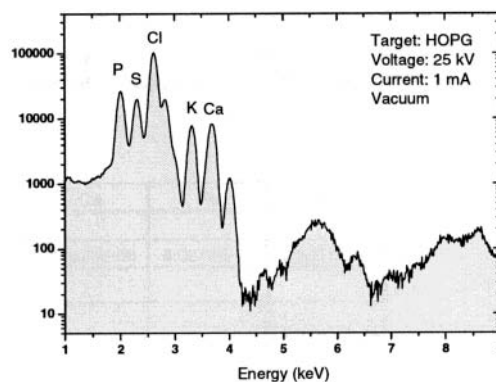


Figure 4. Condition 2: Example of spectrum obtained for P, S, Cl, K, and Ca measurement.

migration on the pellet's surface was also observed when an applied pressure was superior to 3 tons.

As ED-XRF was devoted to be installed near the production lines of milk powders, it was decided to follow the influence of the ambient temperature. Two different parameters were studied and compared: temperature of the powder before pelletizing (20 °C and 40 °C), and temperature of the pellet prior to analysis (-20 °C, 20 °C, 30 °C and 40 °C).

No significant differences were observed between results at 20 °C and at other temperatures.

ED-XRF Conditions and Calculations. Table 2 summarizes the conditions used for the ED-XRF measurements; two conditions were required, and Figures 3 and 4 show the resulting spectra.

A consensus situation between an acceptable repeatability of measurement (long counting time) and a realistic delay for near-the-line measurements (short counting time) was found to define the acquisition time. Seven replicates of the same pellet were thus analyzed at during different times. For all the analytes, relative standard deviations

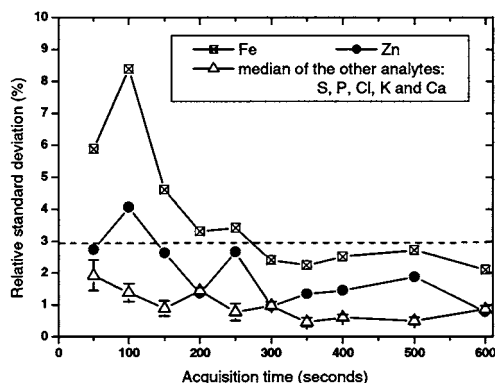


Figure 5. Precision (relative standard deviation) versus acquisition time (number of replicates = 7). From 300 s, relative standard deviations of measurement for both ED-XRF apparatus are lower than 3%. (concentrations: P = 2200 mg/kg, S = 1500 mg/kg, Cl = 10500 mg/kg, K = 6400 mg/kg, Ca = 5300 mg/kg, Fe = 50 mg/kg and Zn = 37 mg/kg)

Table 3. Calculated LOQ (mg/kg) in Milk-based Products with the Spectro Xepos

	LOQ
P	222
S	105
Cl	68
K	1135
Ca	656
Fe	28
Zn	9

Table 4. Robust Relative Repeatability Limits (%)

	P	S	Cl	K	Ca	Fe	Zn
ED-XRF	<8	<10	<9	<4	<5	<11	<7
ref method	<4	<4	<2	<4	<5	<5	<10

of these seven replicates' resulting concentrations were calculated and represented in **Figure 5**.

After 300 s of acquisition, it was found that RSD (%) for all the analytes was lower than 3%. This time was applied during the present study, but an ED-XRF application for a food process control should require a time adjustment based on the production requirements (checks frequency) and the expected method accuracy.

ED-XRF calculations were made using the following formulas, based on Compton's corrections model, implemented in Spectro software: XlabPro 2.3 .

$$V_P = V_{P,0} + \text{slope}_P \cdot C_P + \beta_{Zn,P} \cdot C_{Zn} \cdot C_P$$

$$V_S = \text{slope}_S \cdot C_S + \beta_{P,S} \cdot C_P \cdot C_S + \beta_{Ca,S} \cdot C_{Ca} \cdot C_S + \beta_{Zn,S} \cdot C_{Zn} \cdot C_S$$

$$V_{Cl} = \text{slope}_{Cl} \cdot C_{Cl} + \beta_{K,Cl} \cdot C_K \cdot C_{Cl}$$

$$V_K = \text{slope}_K \cdot C_K + \beta_{Ca,K} \cdot C_{Ca} \cdot C_K$$

$$V_{Ca} = \text{slope}_{Ca} \cdot C_{Ca} + \beta_{K,Ca} \cdot C_K \cdot C_{Ca}$$

$$V_{Fe} = \text{slope}_{Fe} \cdot C_{Fe} + \beta_{Fe,Fe} \cdot C_{Fe} \cdot C_{Fe} + \beta_{Zn,Fe} \cdot C_{Zn} \cdot C_{Fe}$$

$$V_{Zn} = \text{slope}_{Zn} \cdot C_{Zn} + \beta_{Cu,Zn} \cdot C_{Cu} \cdot C_{Zn} + A \cdot C_{Zn} \cdot \ln(C_{Zn})$$

with V ratios fluorescence = intensity/Compton intensity, $\ln C$ = logarithmic correction, and β_{CC} = matrix corrections (only linear/linear).

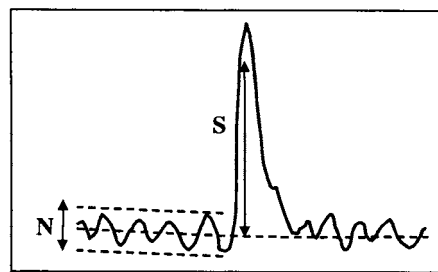


Figure 6. Calculation of the limit of quantification.

Statistical Definitions.

Reference method		y_i
Alternative method (WD-XRF) value		\hat{y}_i
Number of coefficients used in the calibration equations		p
Difference	d_i	$\hat{y}_i - y_i$
Bias	\bar{d}	$\frac{\sum_{i=1}^n (\hat{y}_i - y_i)}{n}$

Standard error of calibration = SEC

$$\sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n - p - 1}}$$

Difference standard deviation = SD(d)

$$\sqrt{\frac{\sum_{i=1}^n (d_i - \bar{d})^2}{n - 1}}$$

Standard error of prediction = SEP

$$\sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n}}$$

Performances of the ED-XRF with Milk-Based Products. Limits of quantification were defined as the lowest amounts of analyte in milk-based product that can be determined with suitable accuracy and precision. Samples used for the determination of limits of quantification were chosen according to the concentration of analyte of interest, so as to be near to the limit of quantification. They were evaluated according to following formula and are listed in **Table 3**.

$$\text{LOQ} = \frac{N \cdot [\text{analyte}]}{S} \cdot 10$$

where N = noise of the instrument, S = signal, and $[\text{analyte}]$ = concentration of the analyte (mg/kg). Robust repeatability limits (r) and robust relative repeatability limits RSD $_r$ (at 95% confidence interval) were calculated. Eleven samples were chosen to cover the entire working range of the seven analytes. Each sample has been measured 15 times. Results are given in **Table 4**. They are compared with reference methods repeatabilities.

RESULTS AND DISCUSSION

Calibrations. Calibrations were established for P, S, Cl, K, Ca, Fe, and Zn. Only concentrations above limits of quantifica-

Table 5. Coefficients Obtained for the Model Corrections

	P	S	Cl	K	Ca	Fe	Zn
intercept	-8.5783 E-03						
slope	3.5614 E-05	6.2517 E-05	9.5396 E-05	5.3844 E-06	8.6833 E-06	6.0578 E-05	2.8344 E-04
$\beta_{Zn,P}$	-5.7595 E-08						
$\beta_{P,S}$		-2.0494 E-12					
$\beta_{Ca,S}$		1.2080 E-09					
$\beta_{Zn,S}$		-1.0173 E-07					
$\beta_{K,Cl}$			1.1615 E-13				
$\beta_{Ca,K}$				3.9017 E-11			
$\beta_{K,Ca}$					-3.3972 E-11		
$\beta_{Fe,Fe}$						-1.3864 E-07	
$\beta_{Zn,Fe}$						2.7147 E-09	
$\beta_{Cu,Zn}$							2.6179 E-06
A_{Zn}							-2.5483 E-05

Table 6. Calibration Data for Milk-Based Products (30 samples)

analyte	min	max	median	samples		SEC (mg/kg)
				no.	R^2	
P	1442	7250	4177	30	0.9833	255
S	1176	2370	1563	28	0.9285	115
Cl	2964	8581	5453	30	0.9719	300
K	4434	12588	7508	30	0.9903	252
Ca	2959	9048	5686	30	0.9813	262
Fe	33.3	115.3	70.6	22	0.9707	4.1
Zn	24.8	72.4	40	30	0.9559	2.7

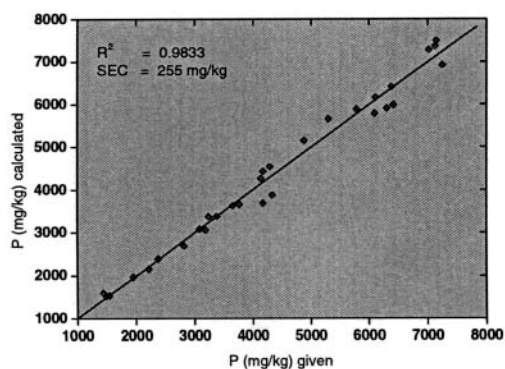


Figure 7. Calibration curve for phosphorus.

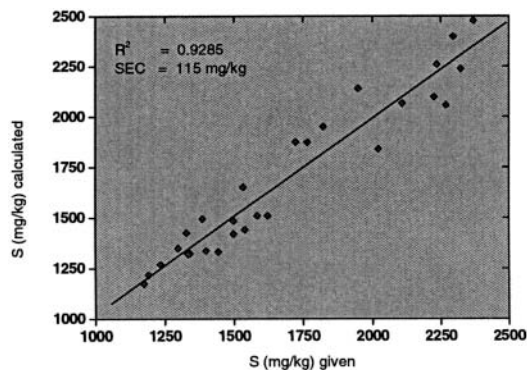


Figure 8. Calibration curve for sulfur.

tion were taken in account. Table 5 gives the resulting coefficients of the Compton model correction. A summary of the statistical treatment of the calibration data is given in Table 6. For each of the following calibrations, each point represents the average of three pellet measurements.

Comparison of the Reference Values and ED-XRF Results. Validation samples were statistically evaluated only if the concentrations given by the reference methods were included

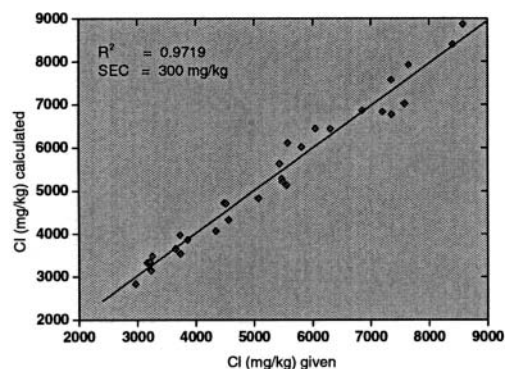


Figure 9. Calibration curve for chlorine.

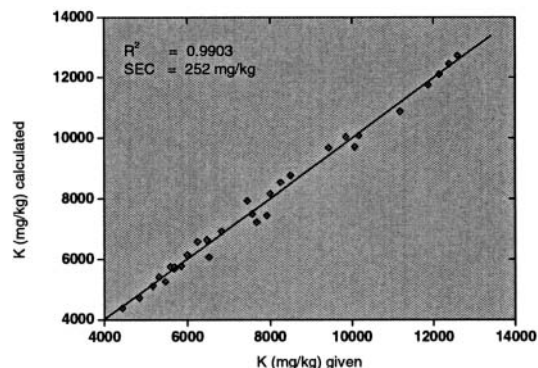


Figure 10. Calibration curve for potassium.

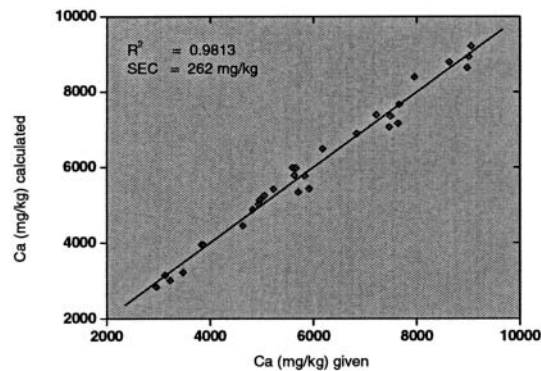


Figure 11. Calibration curve for calcium.

in the calibration ranges. ED-XRF results were compared with reference methods (see Table 7).

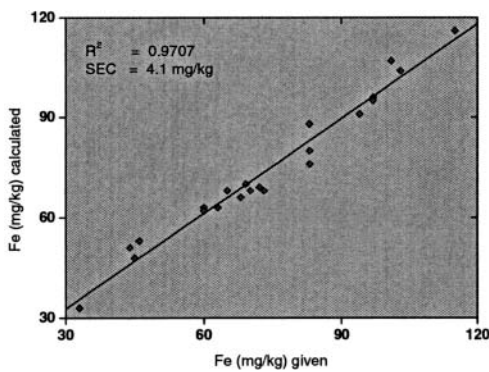
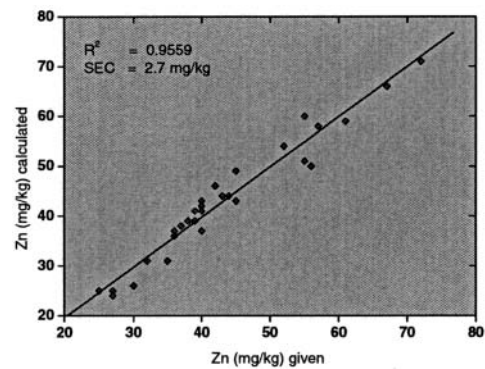
Statistical evaluation of the alternative method performance was performed using robust statistics and formulas described above. The results are given in Table 8.

Table 7. Comparison of Reference Methods (ICP-AES + potentiometry) and ED-XRF Using the Calibration for 30 Validation Samples (unknown)

sample	P		S		Cl		K		Ca		Fe		Zn	
	ref methods concd	ED-XRF concd	ref methods concd	ED-XRF concd	ref methods concd	ED-XRF concd	ref methods concd	ED-XRF concd	ref methods concd	ED-XRF concd	ref methods concd	ED-XRF concd	ref methods concd	ED-XRF concd
U1	4190	3984			5333	5297	7250	6992	5041	4813	60	61	44.7	45.1
U2	4160	4015			3977	4292	6428	6429	5271	5326	98	103	65.1	69.7
U3	1775	1766			2875	2946	5424	5361	3240	3051	64	63	35.6	38.1
U4	1773	1808			3075	3295	5342	5307	3308	3269	36	35	39.5	41.8
U5	4006	4110			4414	4944	6781	7081	5061	5220	66	65	51.8	54.6
U6	4772	5022			5290	5975	8115	8534	5955	6229	86	85	52.2	50.9
U7	7187	6914			8441	8402	12007	11950	8611	8822			27.1	25
U8	2077	2246			2955	3431	4930	5064	3413	3349	66	69	44.6	49
U9	5676	5889			6280	6905	9424	9562	7013	7083	85	79	53.8	52.1
U10	1576	1747			3492	3721	5276	5577	3123	3089	68	71	43.3	47.3
U11	1975	2092	1433	1447	3301	3494	4889	5145	3791	3998	69	68	39.2	41.4
U12	7071	7374			8104	8564			9363	8859			31.8	26.5
U13	1686	1813	1788	1962	3094	3465	5806	6105	3269	3221	29	31	39.4	41.5
U14	4144	4042	1346	1415	4339	4479	6319	6342	5028	5128	86	92	56.7	58.4
U15	7041	7581	2375	2474	7219	7564	11819	11840	9243	8830	95	93	41.7	42.9
U16	2653	2051	1615	1772	3475	3925	5853	6044	4536	4018	60	62	40.6	43
U17	3225	3060	1700	1906	3768	3809	6822	7319	5851	5626	77	81	36.9	36.7
U18	3480	3508	1282	1254	4620	4340	6472	5950	5916	5891	47	53	36.0	32.8
U19	4526	4429	1453	1588	5116	5366	7687	8181	7396	7415	60	70	46.4	50.7
U20	2014	2034	1370	1308	4113	3852	5534	5574	3649	3765	62	63	35.7	38.4
U21	2532	2657	1334	1415	4008	4058	5763	6011	3978	4222	69	71	42.6	45.4
U22	3332	2884	1442	1467	4137	4172	5967	5901	5197	4351	72	68	71.0	70.7
U23	1554	1573	1540	1407	3220	3052	5825	5682	3228	3136	77	71	50.9	52
U24	4126	4026	1553	1511	5350	5047	6966	6871	5426	5418	89	89	61.2	62.7
U25	3722	3584	1391	1381	4902	4694	6833	6712	4787	4649	64	65	37.7	39.9
U26	3944	3820	1464	1436	4868	4644	6598	6253	5193	5046	63	60	38.9	37.5
U27	6063	5831	2121	2068	7020	6891	10039	9608	7639	7099	81	79	56.3	51.3
U28	6064	5860	2213	2163	7448	7313	9983	9840	7503	7212	89	89	40.2	37.2
U29	1635	1669	1782	1780	3215	3327	5488	5443	3042	2913	61	58	36.0	34.9
U30	1605	1672	1580	1564	3008	3039	4865	4922	3119	3121	63	64	38.0	39.8

Table 8. Summary of the Comparison between Reference and ED-XRF Methods

analyte	min	max	median	sample no.	R^2	bias		model ED-XRF = A + B.Reference				SD(d) (mg/kg)	SEP (mg/kg)	SEC (mg/kg)	SEP/SEC
						=0	Yes	A	=0	B	=1				
P	1554	7187	3601	30	0.9844	19	Yes	-35	Yes	1.005	Yes	227	224	255	0.88
S	1282	2375	1540	19	0.9229	-2	Yes	-6	Yes	1.021	Yes	92	94	115	0.82
Cl	2875	8441	4238	30	0.9702	92	Yes	156	Yes	0.994	Yes	311	304	300	1.01
K	4865	12145	6450	29	0.9831	2	Yes	193	Yes	0.978	Yes	327	322	252	1.28
Ca	3042	9363	5051	30	0.9814	-44	Yes	95	Yes	0.963	Yes	261	276	262	1.05
Fe	29.1	97.9	67.2	28	0.9522	0.5	Yes	3.3	Yes	0.961	Yes	3.6	3.5	4.1	0.86
Zn	27.1	71.0	41.2	30	0.9411	1.6	No	-1.0	Yes	1.039	Yes	2.7	2.7	2.7	1.01

**Figure 12.** Calibration curve for iron.**Figure 13.** Calibration curve for zinc.

For all the analytes, A was found to be not significantly different from 0, and B was found to be not significantly different from 1, for the model Alternative = A + B.Reference. This leads to the conclusion that alternatives are similar to reference method results. Only for zinc was the bias found to be significantly different from 0. This shows that the evaluation

of milk-based products using calibrations established with other worldwide products is possible and successful, disregarding origins, recipes, suppliers, and manufacturing processes. Compared to a classical single factory calibration, these kinds of calibration are probably more robust, due to a wider range of calculations and better spread distribution of concentrations (both

due to the diversity of included recipes), ratios of SEP/SEC closer to 1, and the fact that, in most of the cases, bias was found to be lower.

CONCLUSIONS

The Spectro Xepos contains a powerful spectral measurement system, which permits the simultaneous measurement of several elements in milk-based products. The high quality of raw data obtained with this apparatus due to polarization of the X-rays is advantageous compared to other ED-XRF, allowing the lowering of the background interferences. Thus, the effects of differences of composition between recipes (also called matrix effects) are minimized, and all worldwide milk-based products can be included in a single calibration for each element. The ED-XRF technique shows good potential for quality control analyses of P, S, Cl, K, Ca, Fe, and Zn in commercial milk-based products.

Two major elements, sodium and magnesium, occurring in the milk-based products could not be analyzed with this benchtop analyzer, due to insufficient detector sensitivity. Two oligo-elements usually analyzed in milk-based products were not included in this study, because of their low contents in the measured powders, copper (<8 mg/kg) and manganese (<2 mg/kg). At the current time, with present ED-XRF benchtop performances, X-ray Fluorescence spectroscopy could not completely replace an ICP-AES. Use of a liquid nitrogen cooled detector would be necessary and is only available on bigger and more expensive systems.

A key point for a successful application is that a suitable range of products containing a large range of element contents must be available to obtain robust calibrations. The setting up of the calibration standards is a really important step of the installation of XRF in a laboratory. Delays of several months are generally necessary to collect these calibration samples and to complete the reference measurements.

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