Challenge

Quantification of diverse elements ranging from high levels down to trace range in food samples (Ca, Mg, Na, K, Cu, Fe, Mn, Zn, Hg, and As).

Solution

Simple and robust determination using a dual atomizer AAS allowing fast flame AAS and HydrEA coupling for lowest detection limits .

Determination of Macro and Trace Minerals as well as Toxic Trace Metals in Powdered Milk

Introduction

The popularity of milk and dairy products is growing worldwide, resulting in higher milk production and the demand for appropriate quality monitoring to guarantee nutritional value and food safety.

Milk plays an important role in the human diet, from early on with mother's milk in early childhood to other sources of milk and dairy products in later stages of life, in providing healthy and balanced nutrition. Milk is regarded as an important source of diverse macro minerals crucial for healthy growth and for sustaining bodily functions. Calcium, which is strongly associated with milk, for example, is important for bone strength and dental health, but it also plays a role in the functioning of blood vessels and muscles and facilitates blood clotting. Magnesium is important for the transmission of nerve impulses, for blood pressure regulation, or in the synthesis of proteins and DNA. Potassium, which is also found in milk, is important for cardiac function and supports energy production from carbohydrates.

Furthermore, milk provides diverse trace minerals, such as iron, which is a major component in hemoglobin synthesis; zinc, which promotes enzyme-driven processes and plays an important role in the body's immune system; manganese, which is part of many enzymes involved in energy conversion; or copper, which is involved in the formation of red blood cells and plays a role in the function of the central nervous system.

In addition to containing healthy elements, milk may also be contaminated with toxic trace metals, such as arsenic or mercury, which can have adverse health effects and cause cancer or irreversible damage to organs. These elements can originate from contaminated pastures, animal feed, or water.

The following application note describes methods for determining macro minerals, trace minerals, and toxic trace metals in powdered milk, a dairy product used, for example, in manufacturing infant formula, chocolate and other sweets, baked goods, or cheese and yoghurt. It provides the advantages of longer shelf life, no need for refrigeration, and reduced bulk for easier transportation while almost maintaining the content of minerals. Furthermore, lactose powder and whey protein powder were analyzed.

The analysis was performed on a dual atomizer system for AAS, thus allowing simple routine analysis for monitoring the nutritional value using flame AAS as well as special element determination down to lowest detection levels to guarantee food safety by means of graphite furnace technique.

Materials and Methods

Samples and Reagents

- Powdered milk
- Whey protein powder
- Lactose powder

For the analysis using graphite furnace technique following reagents have been used:
Reducing agent: 0.3% $NaBH_4$ / 0.1% NaOH
(2.5 g NaOH and 7.5 g $NaBH_4$ are weighted in a 250 ml glass flask and filled to the mark with deionized water. This solution is stable for about three weeks, and should be stored in a refridgerator. For measurement it is used diluted by a factor of 10).

Carrier solution: 3% HCl
(70 mL conz. HCl, filled to 1000 mL with deionized water)

Sample Preparation

The powdered milk samples were digested completely using the microwave digestion system TOPwave.
Approximately 0.5 g of each sample was weighed and digested (vessel type PM60) with a mixture of HNO_3 and H_2O_2.
Subsequently, the solution was transferred into a graduated flask and filled to a volume of 50 mL with deionized water.

For the determination of Ca, Mg, Na, K, Cu, Fe, Mn and Zn the analysis solutions were diluted with 1.5% HNO_3 and 0.2% $CsCl/LaCl_3$ buffer (for Ca 0.4% $CsCl/LaCl_3$). For measurements of undiluted sample solution, $CsCl/LaCl_3$ buffer was also added.
A certified reference material (milk powder ERM-BD 151) was prepared and analyzed in the same way.
The determination of Zn requires the same HNO_3 concentration for preparation of calibration standards and sample digestion. This allows a compensation of spectral interferences by NO molecule absorbance.

For the determination of Hg the sample solution was measured directly.

For the determination of As, 2.5 mL of each digested sample solution was given into a 25 mL glass flask. In order to remove nitrous gases which may have a signal depressing effect, amidosulphuric acid (15%) was added dropwise (until foaming stopped). Subsequently, a prereduction of AsV to AsIII was performed to allow a determination of the total As content. For this purpose, 2 mL HCl and 2 mL prereduction reagent (5% KI/5% ascorbic acid) were added. The mixture was incubated for 1 hour at room temperature and finally filled to the mark with deionized water (resulting in a dilution factor of 10).

Calibration

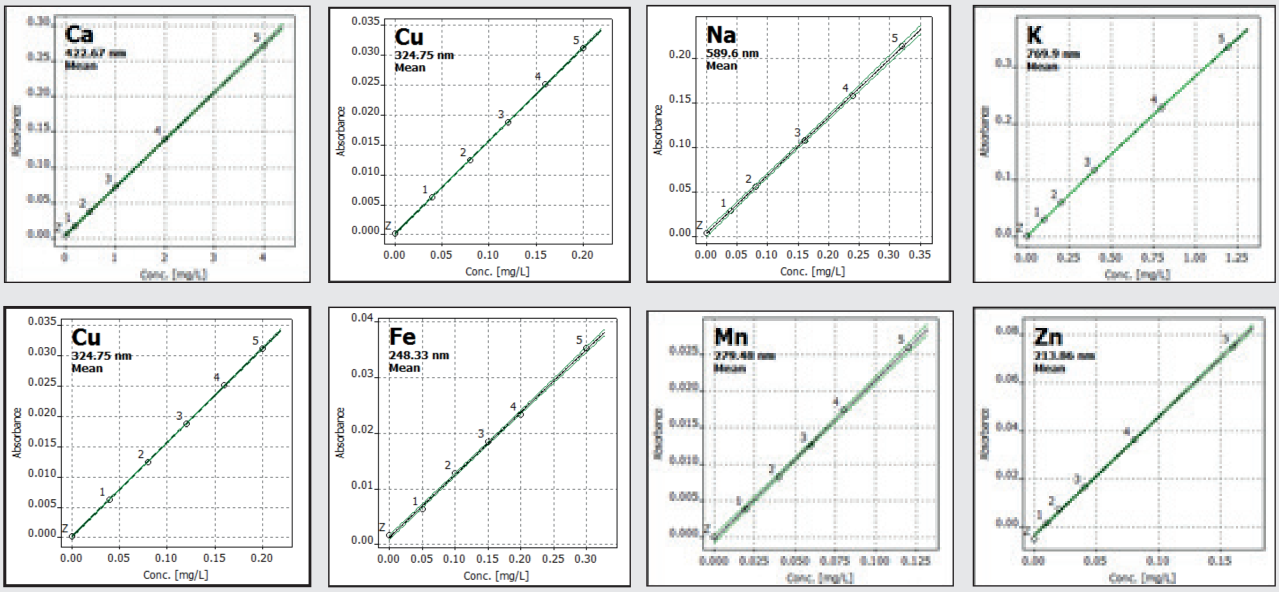
Ca, Mg, Na, K, Cu, Fe, Mn and Zn:

For calibration the standard calibration method was applied. All calibration standards were prepared manually. For calibration of Zn all standard solutions were prepared in the same HNO_3 concentration as applied for the sample digestion and 0.2% $CsCl/LaCl_3$ was added. For all other elements the standard solutions can be prepared in 1.5% HNO_3 and 0.2 % $CsCl/LaCl_3$.

Table 3: Calibration parameters

Standard	Element							
	Ca	Mg	Na	K	Cu	Fe	Mn	Zn
Cal. 0	0	0	0	0	0	0	0	0
Cal. Std. 1	0.2	0.02	0.04	0.1	0.04	0.05	0.02	0.01
Cal. Std. 2	0.5	0.04	0.08	0.2	0.08	0.1	0.04	0.02
Cal. Std. 3	1	0.08	0.16	0.4	0.12	0.15	0.06	0.04
Cal. Std. 4	2	0.12	0.24	0.8	0.16	0.2	0.08	0.08
Cal. Std. 5	4	0.16	0.32	1.2	0.2	0.3	0.16	0.16

Calibration Curves



Figures 1a-1h: calibration curves

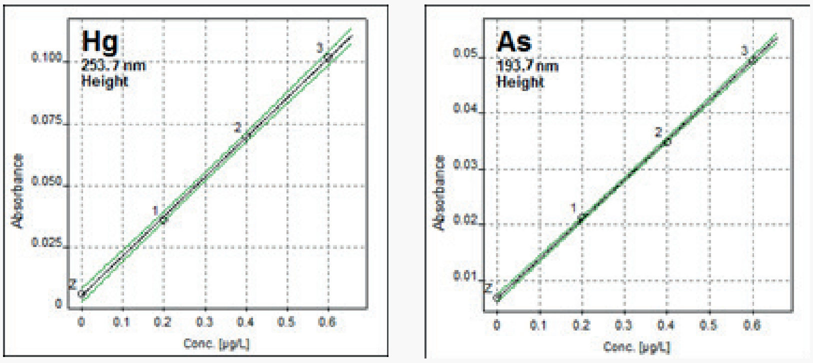
Hg:
The calibration for mercury was performed by using the standard calibration method. The calibration standards were prepared manually analogous to the acid mixture, used for sample digestion, with HNO_3 and HCl.

As:
The calibration for arsenic was performed by using the standard calibration method. The calibration standards were prepared manually analogous to the sample preparation (prereduction)

Table 3: Concentration of standards in µg/L

Standard	Element	
	Hg	As
Cal. 0	0	0
Cal. Std. 1	0.2	0.2
Cal. Std. 2	0.4	0.4
Cal. Std. 3	0.6	0.6

Calibration Curves



Figures 1a-1b: Calibration curves

Instrumentation

The measurements were performed on the novAA® 800 D.
Ca, Mg, Na, K, Cu, Fe, Mn and Zn were determined in flame mode applying a 100 mm burner head, the injection switch SFS 6.0 and an autosampler with automatic dilution function.
As and Hg were determined in graphite furnace mode using the HydrEA technique coupling a hydride system HS60 modular (continuous mode) to the graphite furnace. The graphite tube was coated with gold (Hg) or Ir (As), respectively.

Method settings

The following method settings were used to determine the requested elements.

Table 2: Method parameters

Element	Wavelength [nm]	Slit [nm]	Lamp current [mA]	Burner with [mm]	Burner angle [°]	Burner height [mm]	Flame type	Fuel gas flow [L/h]
Ca	422.7	1.2	3.0	100	0	7	C_2H_2/air	75*
Mg	285.2	1.2	1.5	100	0	6	C_2H_2/air	65
Na	589.6	0.8	3.0	100	0	6	C_2H_2/air	65
K	769.9	1.2	4.0	100	0	8	C_2H_2/air	70
Cu	324.8	1.2	3.0	100	0	6	C_2H_2/air	60
Fe	248.3	0.5	6.0	100	0	7	C_2H_2/air	80
Mn	279.5	0.2	6.0	100	0	6	C_2H_2/air	75
Zn	213.9	0.8	2.0	100	0	4	C_2H_2/air	60

* Aux. Oxidant (75 L/h)

Element	Wavelength [nm]	Slit [nm]	Lamp current [mA]	Measure time [s]	Tube type	Atomization time [s]	max. T_{Atom} [°C]
Hg	253.7	0.8	3.0	5.0	wall (Au coated)	30	950
As	193.7	1.2	6.0	5.0	wall (Ir coated)	30	2150

Results and Discussion

The following tables show the measurement results achieved using the method parameters mentioned above.
The sample solutions were spiked with a defined analyte concentration (QC spike) in order to check for non-spectral interferences. The recovery rates are given in the tables.
For Ca, Mg, Na, K, Cu, Fe, Mn and Zn a certified reference material was analyzed in addition to the samples.

Table 4: Results

Element	Sample	DF	Concentration	Certified value	RSD [%]	QC ** recovery rate [%]
Ca	Powdered milk	50	498+/-126 g/kg	-	0.9	95.3
	ERM-BD 151 (Ref. material)	50	13.0 ± 0.20 g/kg	13.9 ± 0.7 g/kg	0.2	93.5
Mg	Powdered milk	50	289 ± 10.1 mg/kg	-	0.2	96.7
	ERM-BD 151 (Ref. material)	50*	1.19 ± 0.04 g/kg	1.26 ± 0.07 g/kg	1.2	94.4
Na	Powdered milk	50	262 ± 10.4 mg/kg	-	1.0	97.3
	ERM-BD 151 (Ref. material)	50*	3.97 ± 0.08 g/kg	4.19 ± 0.23 g/kg	0.3	94.7
K	Powdered milk	50	1873 ± 73.9 mg/kg	-	0.2	97.6
	ERM-BD 151 (Ref. material)	100*	17.1 ± 0.32 g/kg	17.0 ± 0.8 g/kg	0.3	101
Cu	Powdered milk	1	< 0.38 mg/kg (LOD)	-	-	92.4
	ERM-BD 151 (Ref. material)	1	5.10 ± 0.43 mg/kg	5.0 ± 0.23 mg/kg	2.4	102
Fe	Powdered milk	1	< 1.20 mg/kg (LOQ)	-	-	92.1
	ERM-BD 151 (Ref. material)	2*	49.2 ± 0.96 mg/kg	53 ± 4 mg/kg	0.7	92.8
Mn	MP_2017_01d	1	< 0.7 mg/kg (LOD)	-	-	98.0
	ERM-BD 151 (Ref. material)	1	< 0.7 mg/kg (LOD)	0.29 ± 0.03 mg/kg	-	-
Zn	Powdered milk	1	< 0.33 mg/kg (LOD)	-	-	92.6
	ERM-BD 151 (Ref. material)	1*	43.2 ± 0.94 mg/kg	44.9 ± 2.3 mg/kg	0.2	96.2

DF: manual dilution factor, if the calibration range was exceeded a further dilution (*) was performed by the autosampler
LOD: Limit of detection; determined by 11 times measurement of the reagent blank, calculation for 0.5 g sample and 50 mL volume
LOQ: Limit of quantification
** QC recovery of a certified reference material or a defined analyte concentration, which was spiked to the sample

Table 6: Measurement results and QC recovery

Element	Sample	DF	Concentration [mg/kg]	QC spike concentration [µg/L]	QC spike recovery rate [%]	LOD* [µg/L]
Hg	Lactose powder	1	< 0.002	0.2	93.0	0.02
As	Whey protein powder	10	< 0.01	0.6	95.0	0.01

*LOD: Limit of detection; determined by 11-fold measurement of the reagent blank, calculation for 0.5 g sample and 50 mL volume, including dilution factor for As caused by the pre-reduction step

Conclusion

The novAA® 800 D provides an easy to use and robust instrument for the determination of macro and trace minerals as well as toxic trace metals in food samples. The recovery rates of 92.1–108% for the certified reference material (milk powder ERM-BD 151) demonstrate the reliability of the method applied. Recovery rates of 92.1–98.9% for the QC spikes show the independence of the measurements from the sample matrix.

Using the HydrEA technique allows for efficient analysis of hydride forming elements, as it combines a complete separation of the sample matrix with the high sensitivity of the graphite technique. This enables interference-free determination of Hg and As in the samples given.

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