Chapter 7

Mineral Profile

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Abstract

In recent years, there has been an increase in consumer interest in nutritional and health aspects, which has affected the consumption of animal products. Meat is a source of essential minerals for humans, among which iron stands out, as well as others, such as zinc, phosphorus, magnesium, and potassium. Moreover, meat products can contain high levels of NaCl, and this can be harmful to health. Therefore, it is very important to have an appropriate method for a correct determination of the mineral content in meat and meat products.

Although a wide variety of analytical methods are available for the analysis of minerals and trace elements in food products, Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) is one of the most used techniques, as it allows the simultaneous analysis of a large number of elements.

Taking this into account, this chapter is intended to describe in detail a procedure for mineral analysis of meat and meat products, including determination of total ash and analysis of mineral content by ICP-OES, so that it can be reproduced by other researchers.

Key words Mineral content, Meat, Calcium, Sodium, Iron, Magnesium, Phosphorous, Food analysis, Inductively coupled plasma optical emission spectroscopy (ICP-OES)

1 Introduction

In the last few decades, nutritional habits have notably changed. Specifically, meat consumption has been on the rise since the 1960s in most of countries, but especially from 1980 to the present [1–4]. Moreover, a trend has been observed in recent years: consumers concern for their health and their interest in the nutritional aspects of food has increased, which is also reflected in the consumption of meats and meat products [4, 5].

Meat provides important nutrients for the human diet, mainly proteins, vitamins, and minerals. Minerals have very varied

José Manuel Lorenzo et al. (eds.), *Methods to Assess the Quality of Meat Products*,
Methods and Protocols in Food Science, https://doi.org/10.1007/978-1-0716-2002-1_7,

functions in our body, such as electrolytes, as enzymatic constituents and as building materials (in bones and teeth). They are divided into macro minerals, trace elements (microminerals), and ultra-trace elements. The main elements (Na, K, Ca, Mg, Cl, P, S) are essential for humans in amounts >50 mg/day and trace elements (Fe, I, F, Zn, Se, Cu, Mn, Cr, Mo, Co, Ni) are essential in concentrations of <50 mg/day. Health authorities in most countries have established recommendations for daily intake levels of these essential minerals [6–8].

The mineral content in meat depends on factors like the species, breed, rearing practices, feeds composition, slaughter age, and the cut [8, 9]. According to Hermida et al. [10], the macro minerals and trace elements concentrations in tissues depend on the type of cut, the age of the animals, and other factors. Greenfield and Southgate [11] concluded that the lean/fat tissue ratio affects the levels of most nutrients, which are distributed differently in the two fractions. In addition, thermal processes can change the mineral content of meat [12].

One of the most relevant minerals in meat is iron as it is highly bioavailable. The form in which this iron is found (mainly in heme form, which is bound with myoglobin and hemoglobin) allows 20–30% of it to be absorbed. In addition, the presence of meat favors the absorption of iron forms from vegetables [13]. Another important contribution of meat is zinc as well as magnesium, potassium, copper, and other minerals and microelements [14].

On the other hand, meat products (especially dry-cured products like ham, sausages, and bacon) may contain high concentrations of sodium chloride (NaCl), since salting with NaCl is widely employed to preserve these products, contributes to achieve a characteristic flavor, gives microbial stability and improves proteins solubility [15–17]. However, an excessive consumption of salt is not advisable. Several researches have revealed that high levels of NaCl intake can raise blood pressure and promote certain diseases, which is why it is currently intended to offer consumers healthier food products with low salt content and without losing quality [15, 16]. Potassium chloride is the most common salt substitute in meat products, although calcium and magnesium chlorides also can be another alternative [17].

Considering this, it seems clear that it is important to develop a suitable method for determining the mineral content in meat and meat products.

Nowadays, there are a great variety of analytical methods for the analysis of minerals and trace elements in food. The most commonly used methods include: spectrophotometry, fluorometry, atomic absorption spectrometry (AAS), flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), hydride generation atomic absorption spectrometry (HGAAS), inductively coupled plasma optical emission spectroscopy (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS). The choice of analytical method generally depends on available instrumentation, laboratory experience, and analyte concentration levels [18].

Among all techniques, the use of inductively coupled plasma optical emission spectroscopy (ICP-OES) to identify and quantify minerals in meat and meat products is frequent. It is based on the measurement of the radiation of the spectral line emitted by excited atoms in an Ar plasma generated by inductive heating with a high-frequency electromagnetic field.

ICP-OES is one of the most widely used techniques around the world to determine elements in a wide variety of samples (which must be previously digested) as it allows the simultaneous analysis of a large number of elements [18, 19].

The method we have developed includes two main steps: (1) total ash determination, followed by (2) mineral content determination by ICP-OES.

With all the above, the present book chapter aims to clearly and completely describe a procedure for determining minerals elements in meat and meat products.

2 Materials

Prepare all solutions using mili-Q water and analytical grade reagents, and store stock and standard solutions at refrigerate temperature (4 °C) (*see* **Note 1**).

Follow all waste disposal regulations when disposing of waste materials.

2.1 Total Ash and Mineral Content Determination

Analytical balance (*see* **Note 2**), porcelain crucibles, desiccator for storing them, and a muffle furnace for incinerate samples are needed for total ash determination.

For mineral content determination, these are required: 110 mm quantitative paper filters (FILTER-LAB, Barcelona, Spain), plastic funnels, racks and plastic tubes for ICP-OES autosampler.

1 M HNO_3 solution is required. For this, mix 69.23 mL of nitric acid (65%) (Suprapur or Ultratrace grade) with 900 mL of miliQ water.

2.1.1 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

The quantification of mineral elements is performed using a *PerkinElmer Avio 200* optical emission spectrometer (PerkinElmer, Massachusetts, USA) with an autosampler (S23, PerkinElmer).

The main components of an ICP-OES instrument are the plasma torch, the nebulizer, and the polychromator. The torch consists of three concentric quartz tubes surrounded by a watercooled induction coil connected to a high-frequency generator. Plasma is created by making Ar conductive by exposing it to an electrical discharge that creates electrons and ions. Under the influence of the high-frequency electromagnetic field, the charged particles heat the argon until the plasma reaches temperatures above 9700 °C. This leads to almost complete vaporization of the analyte and high atomization efficiency. The sample is introduced through the nebulizer into the torch using a transporter flow of Ar [18].

ICP-OES is also equipped with a radio frequency source set of 27.12 MHz, a peristaltic pump, a spraying chamber, and a concentric spray nebulizer (MEINHARD glass nebulizer, type K1), using 99.996% liquid argon plasma gas (Praxair, Madrid, Spain).

A 2% nitric acid solution is needed for equipment cleaning between sample measurements.

2.1.2 Calibration

Stock solutions at 1000 mg/L for Ca, K, Mg, Na, P, Fe, Mn, Zn, and Cu (SCP-SCIENCE, Courtaboeuf, France) were used for preparing the standard solutions in 1 N HNO_3, v/v.

The concentration ranges of the standard solutions were: 5–40 mg/L for Ca and Zn, 0.5–2.5 mg/L for Cu and Mn, 2–15 mg/L for Fe, and 50–200 mg/L for K, Mg, Na and P (*see* **Note 3**). Both, final concentrations (mg/L) of each mineral and the volume (μL) of each stock solution (1000 mg/L) used for the preparation of 50 mL of each standard are those listed in Tables 1 and 2 (*see* **Note 4**).

Table 1
Concentration ranges of the standard solutions (prepared in 1 N HNO_3)

	Mineral standard (mg/L)[a]				Adjust to 50 mL with HNO_3 (1 N)[b]			
	Std1	**Std2**	**Std3**	**Std4**	**μL (Std1)**	**μL (Std2)**	**μL (Std3)**	**μL (Std4)**
Ca	5	10	25	40	250	500	1250	2000
Cu	0.5	1	1.5	2.5	25	50	75	125
Fe	2	5	10	15	100	250	500	750
K	50	100	150	200	2500	5000	7500	10,000
Mg	50	100	150	200	2500	5000	7500	10,000
Mn	0.5	1	1.5	2.5	25	50	75	125
Na	50	100	150	200	2500	5000	7500	10,000
P	50	100	150	200	2500	5000	7500	10,000
Zn	5	10	30	40	250	500	1500	2000

Std. standard

[a] Final concentrations (mg/L) of each element in the standards used for calibration

[b] Volume (μL) of the stock solutions (1000 mg/L) used for the preparation of 50 mL of each standard

Table 2
Sodium concentration ranges of the standard solutions (prepared in 1 N HNO_3)

	Na standard (mg/L)[a]						Adjust to 50 mL with HNO_3 (1 N)[b]					
	Std1	Std2	Std3	Std4	Std5	Std6	μL (Std1)	μL (Std2)	μL (Std3)	μL (Std4)	μL (Std5)	μL (Std6)
Na	50	100	150	200	500	1000	2500	5000	7500	10,000	25,000	50,000

Std standard

[a] Final concentrations (mg/L) of sodium in the standards used for calibration

[b] Volume (μL) of the Na stock solution (1000 mg/L) used for the preparation of 50 mL of each standard level

3 Methods

Three-gram (3.00 ± 0.05 g) samples are weighed into porcelain crucibles for mineral analysis. Afterward, the samples are incinerated in a furnace at 450 °C for 12 h (using the protocol ISO 936: 1998 [20]). Then, the crucibles are stored at room temperature in the desiccator until constant weight. The amount of ash obtained is calculated by the difference between the crucible with the sample after incinerating—empty crucible. The ash obtained is dissolved in 10 mL of 1 M HNO_3 and filtered to plastic tubes (Fig. 1).

The next step is the mineral content determination using inductively coupled plasma optical emission spectroscopy (ICP-OES) in order to determine the concentration of Ca, K, Mg, Na, P, Fe, Mn, Zn, and Cu following the protocol defined by Lorenzo et al. [21] with modifications (*see* **Note 5**). Operating conditions of the ICP-OES equipment are: vertical plasma, argon pressure: 6.5 bar, reflected power: 1400 W, sample flow rate: 1 mL/min, nebulizer gas flow: 0.7 L/min, auxiliary argon flow: 0.2 L/min, main argon flow: 10 L/min, purge gas flow: 1.5 L/min, reading time: 2 seconds, height of vertical observation: 15 mm and radial torch configuration.

The operating wavelengths are: Ca, 317.933 nm; K, 404.721 and 766.490 nm; Mg, 279.077 and 285.213 nm; Na, 589.592 nm; P, 213.617 nm; Fe, 238.204 and 239.562 nm; Mn, 257.610 nm; Zn, 206.200 and 213.857 nm; and finally Cu, 324.752 and 327.393 nm (*see* **Note 6**).

Figure 2a and b shows the calibration curves obtained for Ca, Cu, Fe, Mg Na, Zn, Mn, and P at different operating wavelengths (*see* **Note 7**).

Syngistix ICP software is used to control the system and acquire the data. The external standard procedure is used to determine the concentration of each mineral (*see* **Note 8**).

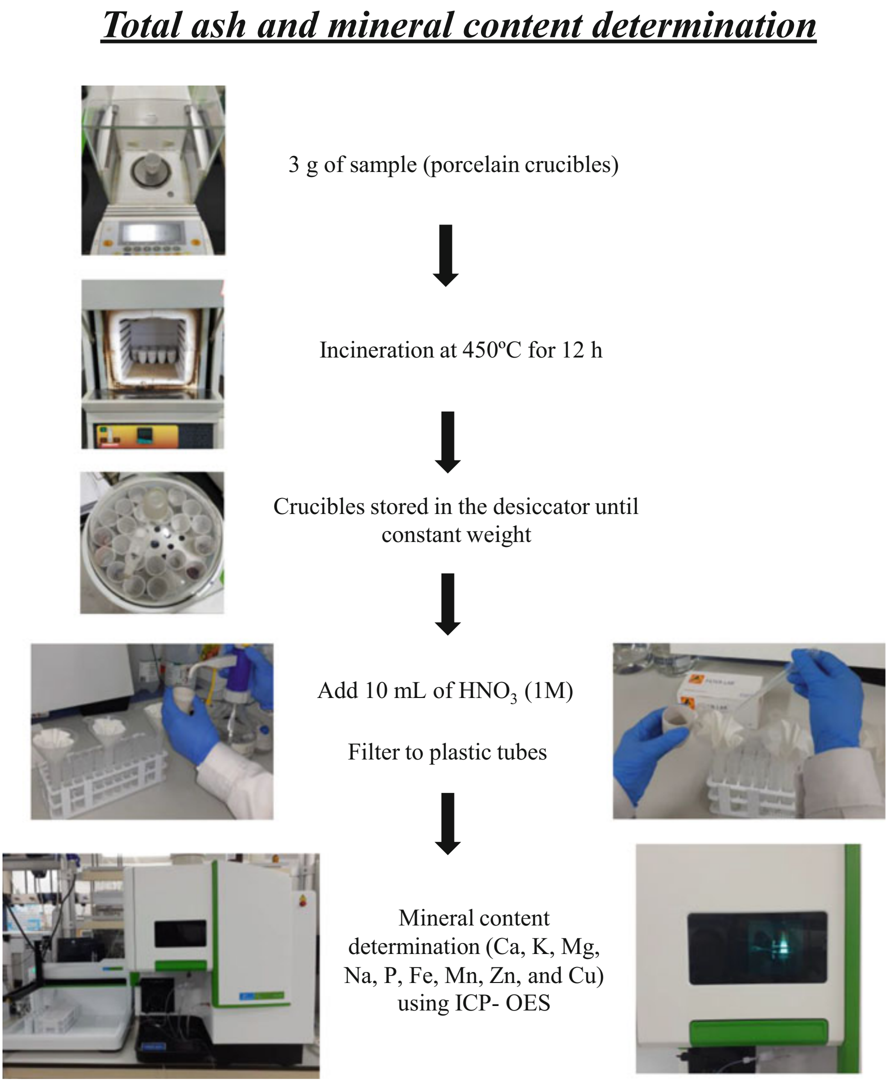

Fig. 1 Schematic and simplified representation of the steps of the total ash and mineral content determination

An example of the spectra obtained for Ca, Mg, and Na at the corresponding wavelength in a meat product sample is shown in Fig. 3. The final value for each element is obtained by calculating the average of two determinations using the Eq. 1, and the results are expressed as mg/100 g of meat.

$$\text{Sample mineral content}\left({}^{\text{mg}}\!/_{100\ \text{g meat}}\right) = \frac{\text{ICP mineral content}\left(\frac{\text{mg}}{\text{L}}\right)}{\text{Fresh sample weight (g)}} \quad (1)$$

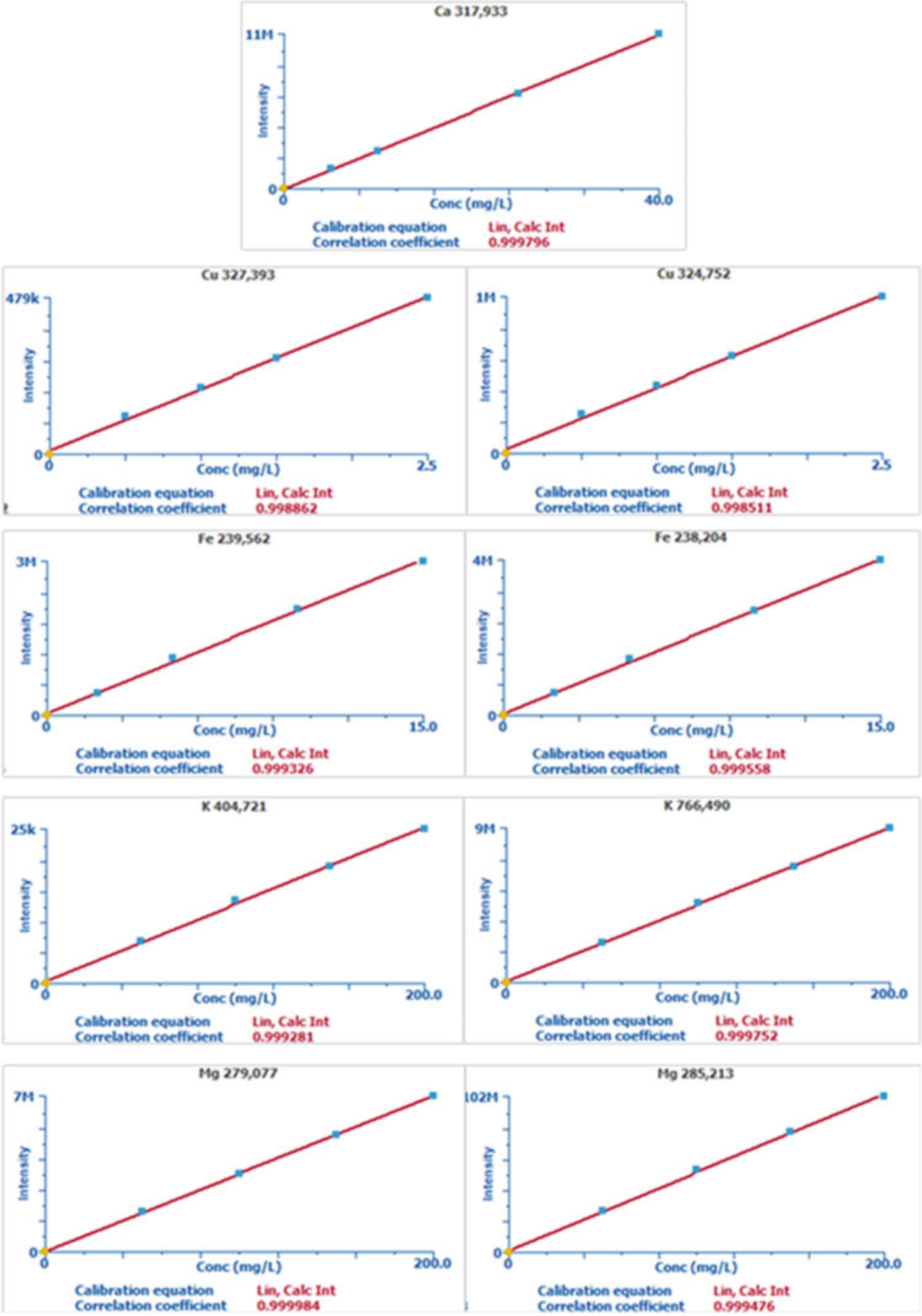

Fig. 2 (**a**) Calibration curves for Ca, Cu, Fe, and Mg at different operating wavelengths. (**b**) Calibration curves for Na, Zn, Mn, and P at different operating wavelengths

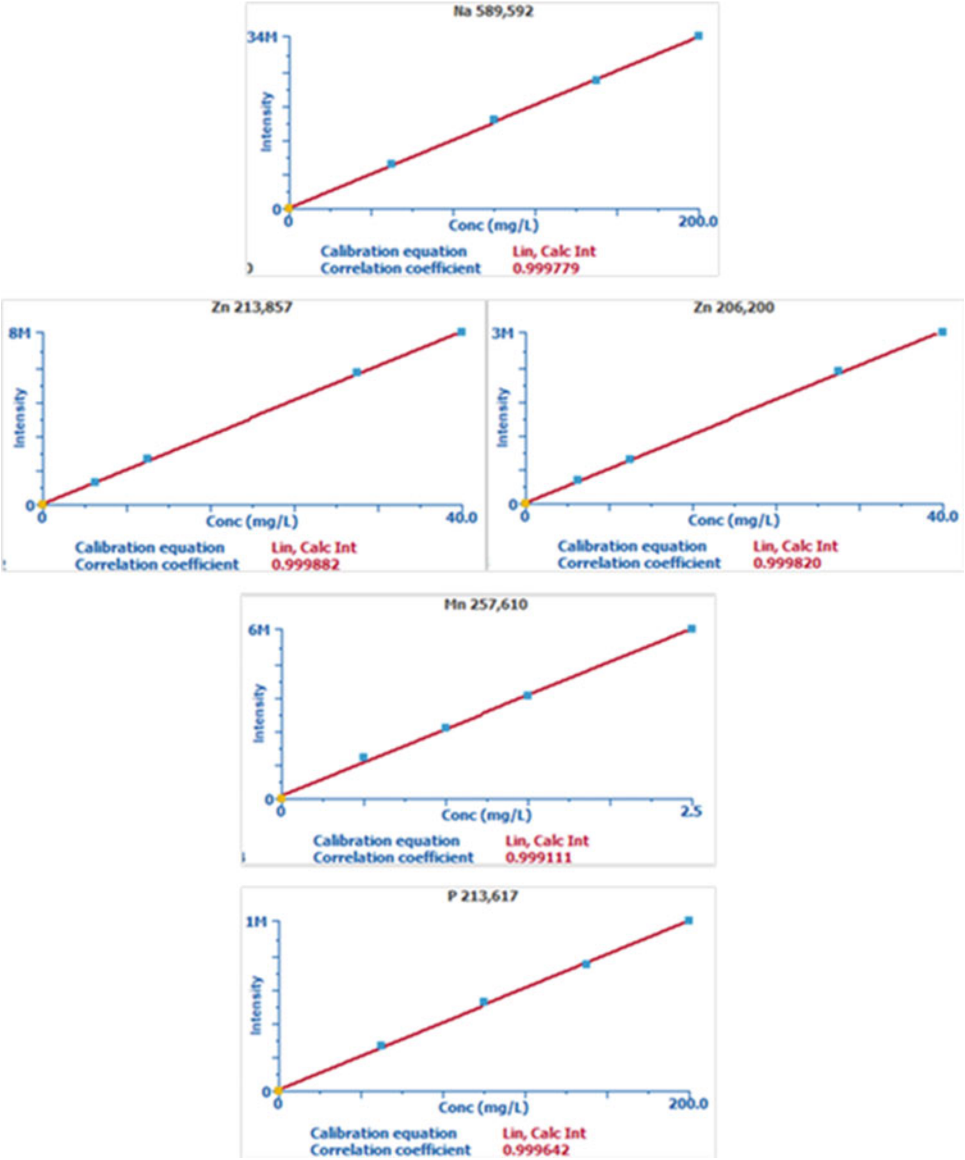

Fig. 2 (continued)

4 Notes

1. Due to the corrosive nature of the reagent used, it is necessary for the operator to take the necessary protective measures (plastic gloves) as well as to carry out all the operations in laboratory fume hoods.
2. An analytical balance with 0.1 mg resolution or higher should be used to weigh the porcelain crucibles.

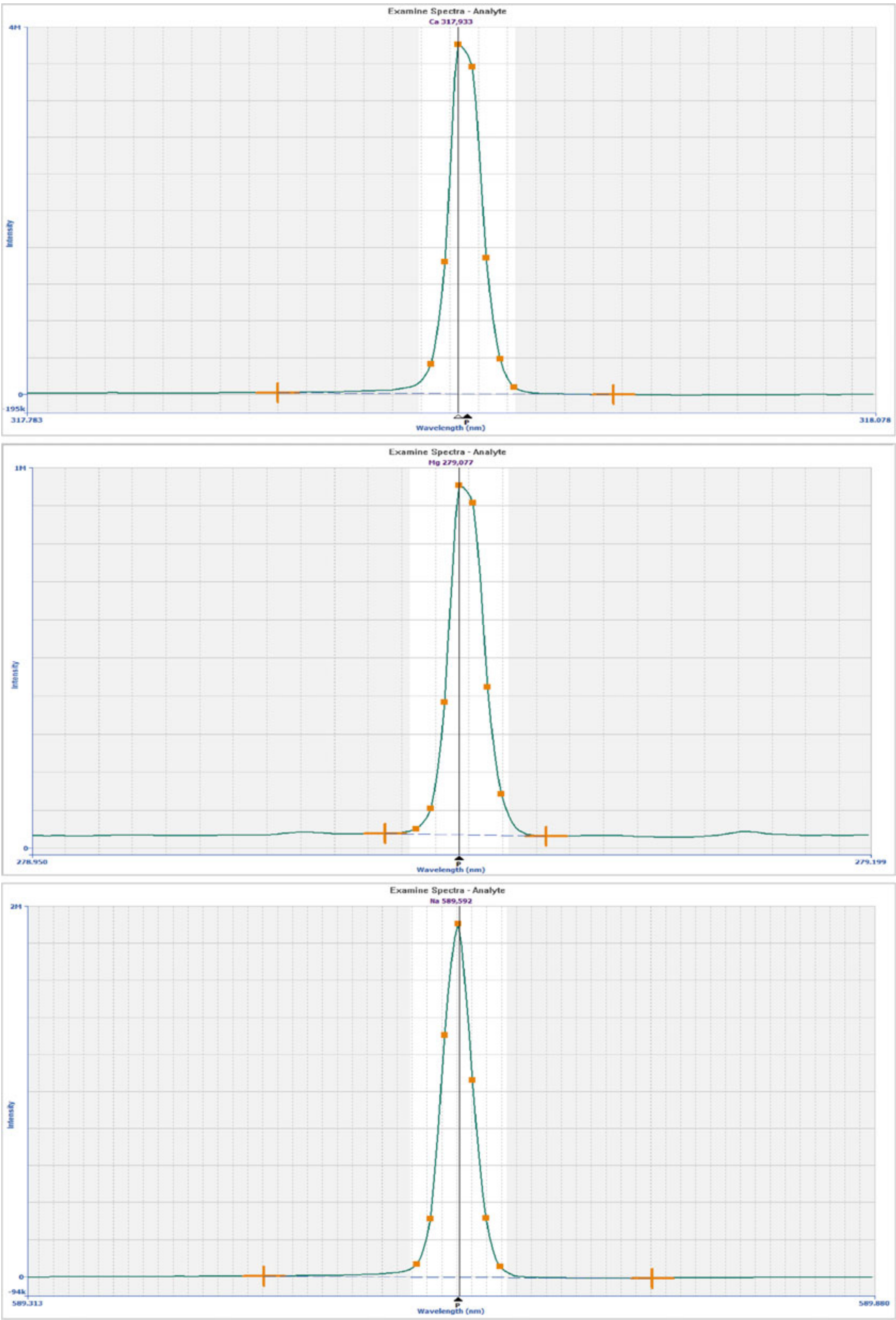

Fig. 3 Spectra for Ca, Mg, and Na at corresponding wavelength in a meat product sample

3. Due to the extremely small quantities (few μL) needed to prepare standard solutions for elements like Cu and Mn, the pipetting process must be scrupulously careful (Tables 1 and 2).
4. Stock solutions and standards prepared for calibrations can be stored refrigerated (4 °C).
5. Standard solutions and samples must be vortexed before measurement.
6. Standards should be injected first, for identification and to create calibration curves for each element at the corresponding wavelength (Fig. 2a, b).
7. It must be verified that there are no interferences that compromise the accuracy of the analytical result. Interferences arise due to differences in composition of the analyzed sample and the external standards and blanks used for calibration.
8. Calibration should be performed every day and/or when any change is made in the conditions of the equipment.

Acknowledgments

This work was supported by Centro Tecnolóxico de la Carne de Galicia. Thanks to GAIN (Axencia Galega de Innovación) for supporting this book chapter (grant number IN607A2019/01). Authors are members of the Healthy Meat network, funded by CYTED (ref. 119RT0568).

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