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Investigation of Sample Preparation Method for the Elemental Analysis of Milk Powder

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INTRODUCTION

Milk powder is a product obtained from the dehydration of liquid milk through a variety of drying process.

The significance of milk powder arose, owing to the fact that liquid milk has a short shelf-life and susceptible to spoilage, especially when it is not stored under the prescribed conditions, or handled properly. Milk is generally abundant in nutrients, and because of its nature it is also an ideal environment for the growth of many microorganisms including ones inducing spoilage (MUEHLHOFF et al., 2013). The transformation of the liquid milk to milk powder paved way to the preservation of the product, allowing easier and more economical bulk transportation and storage conditions, contributing to globalization of milk powder (DIO, 2012).

Whole (full-fat) milk powder and skimmed (non-fat or fat-free) milk powder are the principle types commonly used within the food industry (FOX - MCSWEENEY, 2003). Where among the two types, skimmed milk powder is considered to be more stable due to its fat content being much lower and therefore, less vulnerable to rancidity which is often the reason for the change in the product's odor, palatability, deterioration of nutritional quality and value, and even being unfit for consumption. (ALLEN - HAMILTON, 1994). For this reason, the study will be majorly focused on the optimization of skimmed milk powder.

Aside from being rich in macronutrients, sufficient amounts of vitamins and minerals can also be found in the product. It is a great source of calcium, potassium, phosphorus, and other trace element. A major concern relating to this, is the possible toxic and heavy metal contamination which could arise due to many factors (ASSELT et al., 2016).

Importance of a good analysis must be emphasised especially when concerning matters involving consumers' health and safety. This can be accomplished through proper preparation of the samples by considering the goal of the analysis, sample matrix, analytes, required accuracy and precision, use of internal standards, and analytical instrument to be used. How the sample was prepared prior to analysis leaves a great impact on the data findings. Therefore, observing the effects or its lack of, when the parameters are varied during the sample preparation procedure could be of great interest in terms of evaluating the productivity, effectivity, and efficiency of the method.

1. RAISING A TOPIC

The prevalence of milk powder being used in the industry is due to the fact that, other than being able to avoid early spoilage, it is less demanding in terms of storage and transportation conditions compared to handling liquid milk itself.

One of the main concerns in terms public safety and quality of the product is the possible presence of toxic and heavy metals in the milk powder. Toxic and heavy metal contamination can occur from a wide range of sources, such as the initial quality of the raw milk itself, or from contact throughout the production, processing, packaging, distribution, storage, and transport the product is subject to.

A proper analysis of the milk powder is important to obtain reliable information regarding elemental content of the sample. But in order to achieve a good analysis, a good sample preparation is prerequisite. This initial step is also the most rigorous and critical part of the overall analytical process.

The study's objective serves to find the optimal parameters for the sample preparation technique for the elemental analysis of a powdered skimmed milk and provide an overview of the conditions set, which is most suited to be applied for the examination of a specific element in question. While taking into consideration the principles of green chemistry through the determination of the type of acid to be used for the digestion of the sample, the respective acid's volume to be used, mass of the sample, volume of the hydrogen peroxide added to aid the digestion, time and temperature to be set in the digestion block for both the pre-digestion and main digestion step.

The study will strive to increase the productivity and efficiency of the method by considering the availability of materials and equipment, minimizing quantity usage of sample and reagents, and method simplification considering cost, time, and energy. The goal is to set the parameters that would result to an effective acid decomposition of the samples prior to analysis while preventing the loss of analyte and excessive evaporation of the acids during the heating process in the digestion block. All while concurrently generating consistent, precise, and accurate data fit for the elucidation of the elemental constituents of milk powder.

2. LITERATURE REVIEW

2.1. Milk Powder Introduction

Milk is an excellent source of nutrition. But one of its major drawbacks is that it is a highly perishable product. In history, various attempts were made to prolong the milk's shelf life by preservation and concentration. It was reported that during the thirteenth century, dried milk was carried by the soldiers of the Kublai Khan on excursions, according to Marco Polo. The product was believed to have been made by removing a part of the milk's fat prior to drying while dehydration was achieved through solar heating (CABALLERO et al., 2016). Nowadays, from the total milk production, 15% of which is estimated to be spray-dried (as whole or skimmed milk) for a broad range of applications (FUQUAY, 2011).

Milk powders have been officially defined in the Codex Alimentarius as “milk products which can be obtained by the partial removal of water from milk. The fat and/or protein content of the milk may have been adjusted, only to comply with the compositional requirements in Section 3 of Codex Standard, by the addition and/or withdrawal of milk constituents in such a way as not to alter the whey protein to casein ratio of the milk being adjusted.” (JOINT FAO/WHO CODEX ALIMENTARIUS COMMISSION, 2011).

2.2. Properties of Milk Powder

The product can be categorized into a variety of kind. The different types of milk powder's composition is described in Table 1.

Table 1. Milk Powder Composition

	Types of Milk Powder		
	Whole milk powder	Partly skimmed milk powder	Skimmed milk powder
Milk fat	Minimum 26% and less than 42% m/m	More than 1.5% and less than 26% m/m	Maximum 1.5% m/m
Maximum water ^(a)	5% m/m	5% m/m	5% m/m
Minimum milk protein in milk solids not-fat ^(a)	34% m/m	34% m/m	34% m/m

^(a)The water content does not include water of crystallization of the lactose; the milk solids-not-fat content includes water of crystallization of the lactose.

Source: Codex Alimentarius Commission (2011)

Lipids

Lipids contribute not only as a major energy source for humans but also essential in regards to proper absorption of fat-soluble vitamins due to their sources being only available through diet and cannot be synthesized by the body (ANDRÈS et al., 2024).

As mentioned in Table 1, different types of milk powder exist and can be categorized according to the differences in their fat content, with Whole (full-fat) milk powder and skimmed (non-fat or fat-free) milk powder being the most encountered in the industry, typically having around 26% and 1.5% fat content respectively. Triacylglycerol (TAG) or triglycerides, are the most dominant lipid found in milk. Other categories also present includes diacylglycerols (DAG), sphingolipids, glycolipids, glycerophospholipids, cholesterol, and non-esterified fatty acids (NEFA) and (LIU Et al., 2018). 400 fatty acids were approximated to have been identified by researchers in bovine milk lipids (JENSEN, 2002).

Proteins

High-quality proteins are present in milk powders. In case of bovine milk, the total protein content is approximately around 3.5% by mass, accounts for 38% of the total solid-non-fat content, and responsible for 21% of the energy of whole milk. It is a significant food for the human body the reason being that it contains all 9 essential amino acids (MILLER et al., 2006).

The components comprising milk proteins are as follows: approximately 80% casein and 20% whey protein. Casein, being the most abundant, is responsible for the white and opaque appearance of the milk where: alpha-, beta-, gamma-, and kappa-casein are the key components comprising the division. While whey proteins are present in by-product whey acquired during manufacturing of cheese and casein. It consists of α -lactalbumin, β -lactalbumin, lactoglobulin, glycomacropetides, Immunoglobulin G (IgG), bovine serum albumin and lactoferrin (minor protein) (PATEL et al., 2020).

Carbohydrates

The main carbohydrate in milk powders is Lactose. The total solid-non-fat content accounts for roughly 54% and provides around 30% of the energy of whole milk. Some other carbohydrates i.e. $C_6H_{12}O_6$ (glucose and galactose), sugar phosphates, amino sugars, oligosaccharides (neutral and acidic) and nucleotide sugar are also found in lower concentrations (TOLDRA - NOLLET, 2021).

Vitamins

Nearly all vitamins, at a certain degree, have been detected in milk are essential for the human's body. Vitamins associated with the milk fat include vitamins A, D, E, and K which, therefore, are found in larger amounts in whole milk powder compared to non-fat or skimmed milk powder.

It is also rich in water-soluble vitamins which exist in varying amounts needed by the human body, namely thiamine (vitamin B₁) riboflavin (vitamin B₂), niacin (vitamin B₃), panthothenic acid (vitamin B₅) pyridoxine (vitamin B₆), biotin (vitamin B₇), folic acid (vitamin B₉), cobalamin (vitamin B₁₂), and ascorbic acid (vitamin C). These vitamins are present in certain forms and can be used to fortify milk powders such as in infant formulas (TOLDRA - NOLLET, 2021).

Minerals

Milk powder is rich in minerals. They are often categorized as macrominerals which includes: sodium (Na), potassium (K), chloride (Cl), calcium (Ca), magnesium (Mg), and phosphorus (P); and as trace elements such as: iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), selenium (Se), iodine (I), chromium (Cr), cobalt (Co), molybdenum (Mo), fluoride (F), nickel (Ni), silicon (Si) and boron (B). Most of which are found in milk to some extent in varying concentrations which rely on various factors such as the animals' nutritional and health status, environment, and genes (CASHMAN, 2006).

The amount of heavy metals, elements with high atomic mass and density greater than 5 g/cm³, present in milk is particularly an area of interest. While some are essential to living organisms and required for various biochemical and physiological functions, excess in the quantity also leads to detrimental effects (TCHOUNWOU et al., 2012).

2.3. Presence in the Industry

The nature of milk powders having a low-water activity (a_w) can generally be considered a notable barrier to the growth of unwanted microorganisms, and therefore, improving shelf longevity compared to its liquid form (GURLER et al., 2014). Additionally, the product also poses as a significant ingredient in a variety of dairy and food applications such for cheese, formula for infants, evaporated and/or sweetened condensed milk, ice cream products, pastry products, processed meat and soups, etc. (FOX - MCSWEENEY, 2003).

Globally, the most traded agricultural commodities in terms of dairy products, as percentage of production traded, are full-fat or whole milk powder (WMP) and non-fat/ fat-free skimmed milk powder (SMP) which are also expected to remain being highly traded agricultural commodities in the future (OECD- FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2016). According to Eurostat, 3.0 million tonnes of powdered products were produced in Europe in the year 2022, among which for skimmed milk a total of 20.7 million tonnes were utilized while the whole milk summed to a total of 4.1 million tonnes for the making (EUROSTAT, 2022).

Skim milk powder is claimed to be the most prevalent ingredient used for the manufacture of recombined products of dairy, in comparison to whole milk powders, which are mostly the instant type that are easily dissolve with water for direct consumption and in beverage mixes. Although, concerns limiting its application is due to sensory and quality deterioration caused by being highly vulnerable to oxidative occurrence of the fat in case of whole milk powders (CABALLERO et al., 2003). Therefore, because of the higher fat content, its shelf life is much shorter in comparison to skimmed milk powder which can last up to three years on an average when appropriately stored according to required state and avoid from being subjected to moisture and humid condition (HOPPE et al., 2008).

2.4. Concerns regarding safety of milk powder

Presence of physical, chemical, and/or microbiological toxins may occur in milk powders which is a major concern regarding consumers' health and safety. Elements which poses a huge risk such as arsenic, cadmium, lead, and mercury are particularly monitored due to its toxic nature which can cause adverse effects in human health. While other elements (heavy metals), which are normally present in milk at certain concentrations, must also be taken into consideration as they may evolve into a threat at elevated concentrations (QIN et al., 2009).

According to the Rapid Alert System for Food and Feed (RASFF), an information notification for follow-up was made due to mercury being detected in skimmed milk powder originating from Ukraine. The notifying country was Hungary, notified on the 29th of May 2024. The risk decision was considered potentially serious (EUROPEAN COMMISSION, 2024).

Contamination of heavy metals in powdered milk can take place anytime during processing through contact with the equipments, packaging, storage, and transport (CASHMAN, 2006). It can also be attributed to the quality of raw milk which is affected by environmental condition.

Pollution greatly contributes to the problem by, for instance, its impact on the quality of soil which affects the quality of feed resulting in compromised product containing toxic metals and pesticide (NĂSTĂSESCU et al., 2020). Methods and technologies are constantly being improve and develop to ensure safety of usage and consumption of the product.

2.5. Sample Preparation Techniques

As a means to acquiring results with defined accuracy and precision, it is important to perform an appropriate sample preparation method for the elemental analysis of milk powders.

Techniques for the preparation of a sample can be divided into dry and wet decomposition. Dry ashing is often used to analyze non-volatile metals in samples with large organic matter content. It is a simple technique which involves using a crucible where the samples are held which is then subjected for heating through a furnace. Though its drawback, as in any open system method, is the possible loss of some elements by volatilization and contamination (MITRA, 2003).

In case of the wet process, acid decomposition is one of the most used sample preparation for organic samples. This involves using oxidizing agents, usually concentrated acids such as nitric acid (HNO_3), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), phosphoric acid (H_3PO_4), and hydrogen peroxide (H_2O_2), or a mixture of such reagents are typically used. Heating is then applied on the sample which can be carried out in open or close system (OLIVEIRA, 2003).

Open system are conducted under normal atmospheric conditions using digestion tubes and heat. Open system schemes include: thermally convective wet digestion, where a common heating source is typically involved like Bunsen, heating plate/block, sand bath, etc.; microwave heating/ microwave-assisted wet digestion, where microwaves serves as the energy source for the decomposition which takes place inside the mixture improving both time and efficiency of decomposition; ultraviolet digestion which is commonly utilized with partially or uncontaminated natural water matrices. A few drawbacks include that they may require high temperature while simultaneously taking into consideration the appropriate acid and/or acid mixture's boiling point, as it shall not be exceeded during the process, it also needs high purity reagents, take long process times, and be susceptible to impurities and loss (MATUSIEWICZ, 2003).

Closed system, on the other hand, where samples are digested at increased temperature and pressure are considered to be a huge advantage as less of the reagents volume are required and the time of decomposition is decreased for the digestion (CASTRO et al., 2009). It also contributes to minimizing contamination. Low-pressure or high-pressure vessels are typically used for closed system microwave acid digestion, with liners and caps made from Teflon or perfluoroalkoxy (PFA) with outer jackets. A disadvantage of a close system could be in terms of cost, as conducting a conventional open vessel heating system method is much preferred due to low equipment and operational cost, it also allows higher number of sample to be prepared at the same time compared to that of close systems such as microwave techniques. It can be mentioned that knowledge of the instrument and experience is essential for the operation of working with microwave techniques (UDDIN et al., 2016).

Preparation of milk powder samples prior to its analysis can be executed in a variety of manner according to existing studies. In case of mild sample preparation, methods such as acid extraction, alkaline solubilization, dilution with tertiary amines, solubilization or dilution, and slurry sampling were carried out. Matrix digestions in both open and closed systems were made. While microwave-assisted procedures like microwave-assisted digestion and extraction, focused microwave-induced combustion, microwave-assisted high-pressure flow digestion, microwave-induced combustion, and single reaction chamber microwave-assisted digestion were also performed on milk powders (SOARES et al., 2023).

Table 2 exhibits a collection of different sample preparation for matrix digestion at open systems, where the milk powder is associated with different parameters for its sample preparation process in case of each study.

Table 2. Studies regarding milk (liquid and powder) sample preparation at open system

Preparation Technique	Samples	Reagents or solvents	Procedure	Reference
Conventional wet digestion	Liquid and powdered milk (whole) and milky drink		1.0 mL of sample + 2.0 mL of 14 mol/L HNO ₃ + 1.0 mL of 30% H ₂ O ₂ ; digestion in a heat block at 130°C for 2 h; dilution to 15 mL with water	(RIBEIRO SANT'ANA et al., 2021)
	Liquid milk and milk powder (whole, skimmed and semi-skimmed)	HNO ₃ and H ₂ O ₂	200 mg of powder sample + 1.0 mL of water (or 1.0 mL of liquid sample) + 1.5 mL of concentrated HNO ₃ ; heating at 110°C for ca. 1 h; addition of 2.0 mL H ₂ O ₂ and heating for 1 h; Dilution to 10 mL or 40 mL with water	(LACERDA SANCHES et al., 2020)
	Powdered milk (whole and skimmed) and Infant formula	HNO ₃ and H ₂ SO ₄	1 g of milk powder + 10 mL acid mixture HNO ₃ / H ₂ SO ₄ (3:1); digester block at 350°C for 4 h with reflux system; filled up to 40 mL with ultrapure water	(ORESTE et al., 2016)
	Powdered milk /Infant formula	HNO ₃ , HClO ₄ and H ₂ O ₂	0.5 g of sample + 15 mL of HNO ₃ then heated to dryness; +5 mL of HNO ₃ and heated again; +5 mL of HClO ₄ heated and evaporated to 1mL; cooled and H ₂ O ₂ was added dropwise until colorless; filled up with 50 mL de-ionized water	(BORKOWSKA-BURNECKA et al., 1996)
Fenton digestion	Breast milk, whole milk and milk formula	H ₂ O ₂ , Fe(NH ₄) ₂ (SO ₄) ₂ and NH ₄ OH	0.5 mL of sample + 50 µL of 1 µg/mL 129I + 0.5 mL of H ₂ O ₂ + 50 µL of 0.1 mol/L Fe(NH ₄) ₂ (SO ₄) ₂ and resting for 15 min, 25 µL of NH ₄ OH and Fenton digestion at 60°C for 12 + hours, dilution to 5.0 mL with NH ₄ OH, vortexing and centrifugation for 15 min at 2°C	(SHELOR et al., 2011)

Source: RIBEIRO SANT'ANA et al., 2021; LACERDA SANCHES et al., 2020; ORESTE et al., 2016; BORKOWSKA-BURNECKA et al., 1996; SHELOR et al., 2011; own edit

Many factors contribute to a good sample preparation. The goal is usually to attempt increase the overall productivity in this process by considering financial aspects, availability of materials and equipment, possibility of miniaturization, sample amount usage, and simplification of the method (SOLTANI, 2014).

In a conventional open wet digestion system, variables to be optimized typically include the digestion reagents, temperature, and time. Sample matrix and elements to be analysed are taken into consideration for choosing the digestion reagents which are usually acids with strong oxidizing capability (MOHAMMED et al., 2017).

Nitric acid (HNO_3) is widely used, due to not only being accessible and affordable, but also for its ability to turn solutes to much higher oxidation states. It can be used by itself or in combination with other acids to suppress the insoluble oxide layer development on the sample surface that hinders further dissolution (UDDIN et al., 2016). Nonetheless, non-oxidizing acid such as hydrochloric acid (HCl) are also used, where materials are dissolved through the formation of stable chloride complexes with dissolving cations. Chlorate, permanganate and hydrogen peroxide are also commonly added to aid in strengthening this oxidizing capability for the digestion. Volume of these reagents, ratio, and concentration can be evaluated for the best results to be used for analysis.

Temperature for digestion is determined by the boiling point of the acids as well as the sample volatility. While digestion time controls the length of exposure of the sample to the acids, assisting in the increase of extent of solubilization (ROBINSON et al., 2014).

2.6. Elemental Analysis

Quality investigation and determination of the quantity of the components found in food samples can be achieved through several methods. Although over the time, there has been a consensus that atomic spectrometry became predominant for the trace elemental analysis in food. This includes flame emission spectrometry (FES) , flame atomic absorption spectrometry (FAAS), and graphite furnace atomic absorption spectrometry (GFAAS) (MCKINSTRY et al., 1999).

For milk and dairy products' determination of elemental composition, atomic and molecular spectrometric techniques have been preferred for the analysis in conjunction with plasma

techniques. Particularly, inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) methods as present in this study.

2.6.1. Inductively coupled plasma optical emission spectroscopy (ICP-OES)

ICP-OES operates by the principle of using a plasma torch surrounded by a cylindrical induction coil connected to a radio frequency (RF) generator. After ignition, energy from the RF generator is passed from the copper coil to the free electrons above creating high energy electrons. A process known as inductive coupling occurs through the collision of these high energy electrons with argon atoms, more free electrons are stripped off forming a high temperature plasma (KOVÁCS - CSAPÓ, 2015).

Information about the sample is obtained through measuring the light induced by excited atoms and ions in the plasma. To separate the polychromatic radiation into individual wavelengths, a monochromator can be used, or a polychromator for several different wavelength at once. For the detection, photo-multiplier (PMT), charge-coupled device (CCD), or charge-injection device (CID) can be applied for the process (BOSS - FREDEEN, 2004).

ICP-OES is one of the widely used techniques for elemental quantification in dairy and milk products in general, due to its low limit of detection (LOD) and great capacity for determination over wide range of concentration, revolutionizing trace elemental analysis (DOUVIRIS et al., 2023).

This analytical technique has been used in several studies involving dairy products, such as for the chemical analysis of the heavy metals: copper(Cu), lead(Pb), mercury(Hg), and nickel(Ni) and their antagonists: iron(Fe), zinc(Zn), calcium(Ca), selenium(Se), and cobalt(Co) in UHT milk for both liquid and powdered products (RIBEIRO et al., 2021), whose sample preparation method was described in Table 2; and quantification of 20 macro and trace elements (Na, K, Ca, Mg, Al, B, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Li, Pb, Zn, Ni, Sr and V) in yogurt samples (LUIS et al., 2015).

2.6.2. Inductively coupled plasma mass spectrometry (ICP-MS)

The principle of ICP-MS is similar to that of the ICP-OES in the initial part. It has a sample introduction system and inductively coupled plasma (ICP), after which there will be an interface (cones), ion optics, mass analyser and detector instead.

In this technique, the plasma, usually out of argon, serves to ionize the sample. The temperature of the plasma and the ionization potential defines the degree of ionization of the elements. Most elements are able to ionize efficiently in the plasma due to their first ionization potential being lower than argon (WILSCHEFSKI - BAXTER, 2019). After going through the ion optics, the ions arrive at the mass analyzer, where their mass-to-charge ratios are the very basis for ion separation, achieved in several ways such as through a quadrupole, magnetic sectors systems, and time of flight (TOF) mass spectrometers. The detector used is often an electron multiplier (EM), where emission of electrons from the surface of the dynode held at negative voltage, are generated upon impact of the positively-charged analyte ions. The emitted electrons are accelerated to the next metal plate inducing secondary emission creating more electrons. The detector converts them to electrical pulses whose magnitude is in accordance to the amount of the inspected sample's analyte ions present (THOMAS, 2003).

An advantage of working with ICP-MS is that, it is more sensitive to elemental trace analysis by offering even much lower detection limits in the sense of parts per trillion (ppt) range compared to that of ICP-OES.

The technique is also often used in dairy studies, such as for the identification of the total content of 45 elements in room temperature acid sonication of milk samples proposed as a fast alternative methodology (CAVA - MONTESINOS et al., 2005).

3. MATERIALS AND METHODS

3.1. Samples and Reagents

The milk powder sample used for this study is the pack of 200 g skimmed milk powder from Tutti Kft. (Figure 1). The chemicals and reagents used for the digestions are Nitric Acid (69 m/m%, Sigma-Aldrich, Merck, Germany), Hydrochloric Acid (37%, BDH Prolabo, VWR Chemicals, France), Hydrogen Peroxide (30%, Darmstadt, Merck, Germany), and deionized water (DI water) with its resistance being 8.2 MΩ cm (MilliQ, Millipore Corp., Bedford, MA,



Figure 1. Skimmed milk powder sample
Source: SPAR (12.06.2024)

USA).

3.2. Sample Preparation Method

Sample Preparations were carried out at the Institute of Food Science of the Faculty of Agricultural and Food Science and Environmental Management of the University of Debrecen.

The milk powder was prepared through wet digestion at atmospheric pressure according KOVÁCS et al., 1996 where a general method for the digestion process was suggested.

The compromised optimum condition proposed for the digestion of sample preparation method involves the following steps: weighing 2 gram of plant sample into a digestion tube and adding 10 cm³ of 65% nitric acid (HNO₃) into it. The sample can then be placed on a heating block at 60°C for 30 min or left at room temperature overnight. 3 cm³ of 30% hydrogen peroxide (H₂O₂) is then added to the sample, then placed back to the heating block at 120°C for 90 min. After

the digestion, the solution is filled up to 50 cm³ with ultrapure water. Afterwards, the sample solution is filtered using MN 640W (or equivalent) filter paper (KOVÁCS et al., 1996)

For the purpose of determining the elemental content of the samples, different parameters must be investigated to find the optimal conditions for the sample preparation process that is suited to be used for this specific product in question. The parameters and their values used for this study is summarized in Table 3.

Table 3. The parameters studied and their values for the open system wet digestion

Category	Value
Type of Acid	HNO ₃ and HCl
Mass of sample	1 g, 2 g, and 3 g
Volume of cc. HNO ₃	5 cm ³ , 10 cm ³ , and 15 cm ³
Temperature for pre-digestion	30°C, 60°C, and 90°C
Duration of pre-digestion	30 min, 60 min, and 90 min
Volume of cc. H ₂ O ₂	1 cm ³ , 3 cm ³ , and 5 cm ³
Temperature for main digestion	100°C, 120°C, and 140°C
Duration of main digestion	60 min, 90 min, and 120 min

Source: own data (2024)

3.2.1. First phase: Optimization of the type of acid

The following acids were studied: nitric acid (HNO₃) and hydrochloric acid (HCl).

The samples were weighed into the heat-resistant digestion tubes with the mentioned mass (1 g, 2 g, and 3 g) 10 cm³ of nitric acid and 10 cm³ of hydrochloric acid were added to the digestion tubes accordingly with triplicate measurements for each mass sample and type of acid combination, then vortexed and left overnight:

- 1 g, 2 g, and 3 g with 10 cm³ of hydrochloric acid (HCl)
- 1 g, 2 g, and 3 g with 10 cm³ of nitric acid (HNO₃)

In the following day, the digestion tubes were placed in the heating block of a LABOR MIM OE-718/A block digestion apparatus for pre-digestion at 60°C for 30 minutes. The samples were removed from the heating block and allowed to cool at room temperature before adding 3 cm³ of 30% hydrogen peroxide to the digestion tubes. The samples were then placed back to the heating block for main digestion which is carried out at 120°C for 90 minutes.

Digested samples were then cooled at room temperature before filling the tubes to 50 cm³ using DI water. The samples were filtered with FILTRAK 388 filter paper using a funnel and collected in centrifuge tubes for analysis.

3.2.2. Second phase: Optimization of the mass of sample and volume of acid used for digestion

The following parameters were studied: volume of acid (5 cm³, 10 cm³, and 15 cm³), and mass of samples (1 g, 2 g, and 3 g). Blank samples were also prepared.

The samples were weighed into the heat-resistant digestion tubes with the mentioned mass (1 g, 2 g, and 3 g) where 5 cm³, 10 cm³, and 15 cm³ of nitric acid was added to the digestion tubes accordingly with triplicate measurements for each mass sample and volume acid combination (Figure 2). The combination of samples made are as follows, then vortexed and left overnight:

- 1 g, 2 g, and 3 g with 5 cm³, of nitric acid (HNO₃)
- 1 g, 2 g, and 3 g with 10 cm³, of nitric acid (HNO₃)
- 1 g, 2 g, and 3 g with 15 cm³, of nitric acid (HNO₃)



Figure 2. Samples prepared for addition of acid
Source: own photo (2024)



Figure 3. Filtering of the samples
Source: own photo (2024)

In the following day, the digestion tubes were placed in the heating block of a LABOR MIM OE-718/A block digestion apparatus for pre-digestion at 60°C for 30 minutes. The samples were removed from the heating block and allowed to cool at room temperature before adding 3 cm³ of 30% hydrogen peroxide to the digestion tubes. The samples were then placed back to the heating block for main digestion which is carried out at 120°C for 90 minutes.

Digested samples were then cooled at room temperature before filling the tubes to 50 cm³ using DI water. The samples were filtered with FILTRAK 388 filter paper using a funnel and collected in centrifuge tubes for analysis (Figure 3).

3.2.3. Third phase: Optimization of the time and temperature for the pre-digestion process

The type of acid, optimal mass, and volume of sample are then decided. Using this information, the time and temperature for the pre-digestion are then changed.

The temperatures investigated were 30°C, 60°C, and 90°C, while the time was tested in 30 min, 60 min, and 90 min. The initial steps were carried out similarly from the previous phase but with the optimized parameters. Only the pre-digestion conditions will be examined. The experiment was done in replicates of 3 for each varied time and temperature combination:

- 30°C for 30 min, 60 min, and 90 min
- 60°C for 30 min, 60 min, and 90 min
- 90°C for 30 min, 60 min, and 90 min

The volume of the hydrogen peroxide and conditions for main digestion remained the same. The samples were then filtered with FILTRAK 388 filters, collected, and analyzed.

3.2.4. Fourth phase: Optimization of the volume of the hydrogen peroxide added

After the best temperature and time for the pre-digestion has been confirmed, the volume of the hydrogen peroxide to be added is then varied (1 cm³, 3 cm³, and 5 cm³) with 3 replicates of each.

The condition for the main digestion remains the same and the samples were then filtered, collected, and sent for analysis.

3.2.5. Final phase: Optimization of the time and temperature for the main digestion process

The final step is changing the condition for the main digestion where the time: 60 min, 90 min, and 120 min and temperature: 100°C, 120°C, and 140°C were investigated with 3 replicates of each combination and the samples were then filtered, collected, and analyzed:

- 100°C for 60 min, 90 min, and 120 min
- 120°C for 60 min, 90 min, and 120 min
- 140°C for 60 min, 90 min, and 120 min

3.3. Instrumentation for analysis

The analysis for the elements were carried out by Thermo Scientific iCAP 6300 Dual view inductively coupled plasma optical emission spectrometry (ICP-OES) and Thermo Scientific X-Series II inductively coupled plasma mass spectrometry (ICP-MS). The main parameters of the equipment for the analysis are listed in Table 4 for ICP-OES and Table 5 for ICP-MS. They were controlled by iTEVA 2.8.0.97 and Thermo PlasmaLab 2.5.10.319 software.

Table 4. Main parameters of ICP-OES

ICP-OES		
RF power		1350 W
Plasma gas flow rate		12.0 L min ⁻¹
Auxiliary gas flow rate		1.0 L min ⁻¹
Nebuliser gas flow rate		1.0 L min ⁻¹
Sample uptake		50 rpm
Plasma view		Axial and Radial
Integration time	low WL	
	range	10s
	high WL	
	range	10s
Repeats		2
Nebuliser		concentric
Spray chamber		cyclonic
Elements and wavelengths (nm)		Al(396.1), As(197.2), B(249.7), Ba(255.4), Ca(422.6), Cd(226.5), Co(228.6), Cr(267.7), Cu(324.7), Fe(238.2), Hg(184.9), K(769.8), Li(670.7), Mg(279.5), Na(588.9), Ni(221.6), P(177.4), Pb(216.9), S(182), Se(203.9), Sr(407.7), Ti(334.9), V(292.4), Zn(213.8)

Source: own data (2024)

Table 5. Main parameters of ICP-MS

	ICP-MS
RF power	1400 W
Plasma gas flow rate	14.0 L min ⁻¹
Auxiliary gas flow rate	0.86 L min ⁻¹
Nebuliser gas flow rate	0.88 L min ⁻¹
Sample uptake	20 rpm
Dwell time	100 ms
Sweep	9
Main run	3
Pole bias	-16.0
Hexapole bias	-10.0
CCT gas	H ₂ -He in 7:93
CCT gas flow rate	6 mL min ⁻¹
Nebuliser	concentric
Spray chamber	conical (2°C)
Analysed masses	¹¹ B, ²⁷ Al, ⁴⁵ Sc, ⁵¹ V, ⁵³ Cr, ⁵⁶ Fe, ⁵⁹ Co, ⁶⁰ Ni, ⁶⁵ Cu, ⁷⁸ Se, ⁸⁸ Sr, ⁸⁹ Y, ⁹⁵ Mo, ¹¹⁴ Cd, ¹³⁷ Ba, ¹³⁹ La, ¹⁴⁶ Nd, ²⁰⁸ Pb
Internal standard	⁷² Ge, ¹⁰³ Rh, ¹⁹⁵ Pt

Source: own data (2024)

3.4. Statistical Analysis

IBM SPSS Statistics V22.0 was used for the statistical analysis. One-way ANOVA and Tukey-test were applied to examine the relationships between parameters and factors. Differences were considered significant at $p \leq 0.05$.

4. RESULT AND EVALUATION

4.1. First phase: Optimization of the type of acid

The initial step involved comparing which type of acid is most suitable for the digestion process. The 1 g, 2 g, and 3 g with 10 cm³ of nitric acid (HNO₃) was compared with 1 g, 2 g, and 3 g with 10 cm³ of hydrochloric acid (HCl). During the first few steps up until the pre-digestion, no major differences were observed for the acids other than the color of the solutions. For the addition of nitric acid, a yellow-orange solution was brought about, wherein throughout the first few minutes of the pre-digestion step, some discoloration on the walls of the tubes took place. While in case of the hydrochloric acid, the color of the solution observed was on the darker shade of blue/violet (Figure 4).

Upon cooling and adding the hydrogen peroxide (H₂O₂), a major difference between two of the acids' effect on the sample was noted. While no abrupt reaction occurred in case of adding the hydrogen peroxide to the samples with nitric acid, the samples with hydrochloric acid displayed violent foaming and its overflow from the tubes was observed after some seconds upon the addition of the hydrogen peroxide (Figure 5).



Figure 4. Left: sample with HCl;
Right: sample with HNO₃
Source: own photo (2024)



Figure 5. Violent foaming upon addition
of H₂O₂ on samples with HCl
Source: own photo (2024)

After the main digestion of the samples, the sample with hydrochloric acid (HCl) turned much darker in color, and left a permanent visible stain on the digestion tubes. When the samples with hydrochloric acid (HCl) was analysed, it was noted that it can cause problems such as staining the tubes of the instrument which might also be a risk of potentially damaging the equipments.

Figure 6 illustrate the difference in color of the filtered samples from the two acid investigated. It can be visually identified as sample with HCl filtrate were black in color in contrast to the yellowish filtrate obtained from using nitric acid (HNO₃).



Figure 6. Filtering of the sample with HCl (left) vs. Filtrate of HNO₃ (right)
Source: own photo (2024)

The result of the elemental analysis is demonstrated in Figure 7. The concentration was plotted against the mass of the sample and its standard deviation was also calculated and indicated in the graph. It can be observed that in case of most elements such as B, Zn, and Cu as illustrated in the figure, the concentration value of the nitric acid (HNO₃) is much higher for majority of the cases tested in different mass sample (1 g, 2 g, and 3 g) than the hydrochloric acid (HCl) where the volume used for comparison was 10 cm³ of the acids.

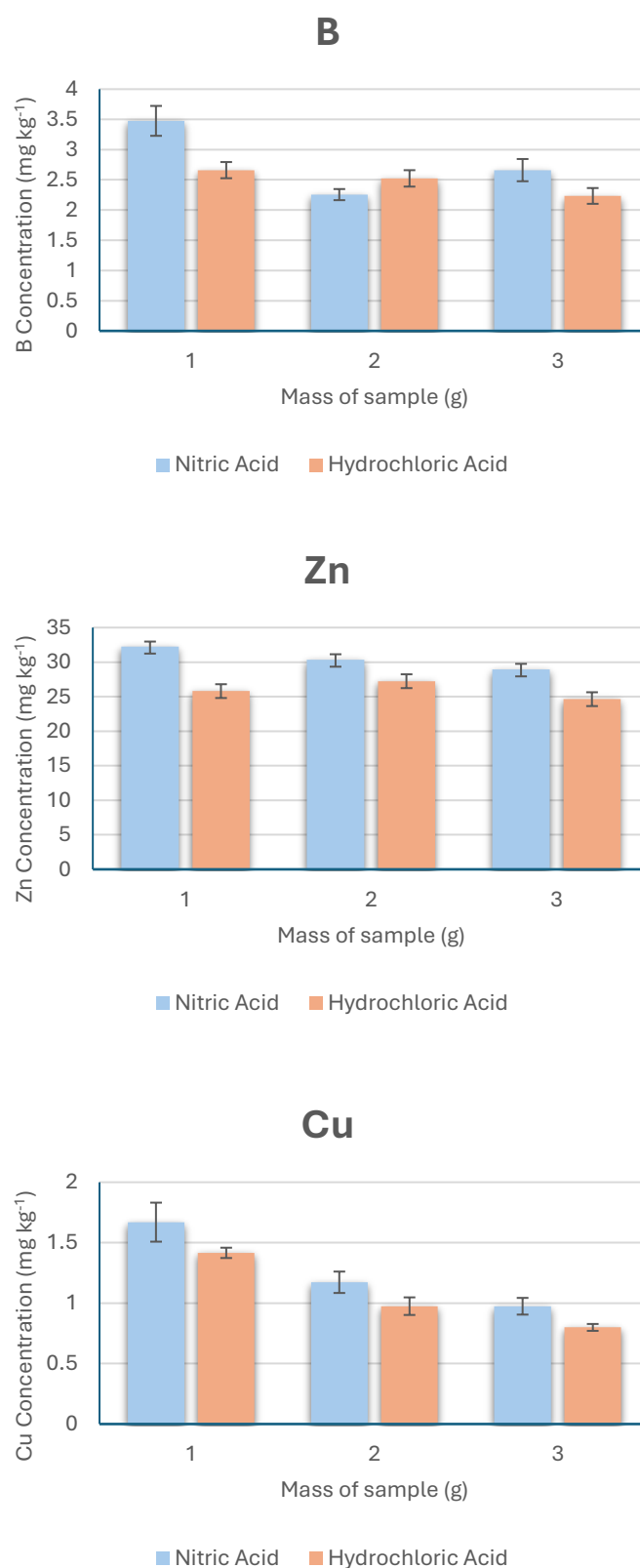


Figure 7. Concentration (mg kg^{-1}) of B, Zn, and Cu against the mass of the sample, in case of optimization of type of the acid to be used in 10 mL volume.
Source: Own data (2024)

According to the findings in this phase, it can be presumed that the nitric acid (HNO_3) is a more befitting option as the type of acid to be used for the digestion method as opposed to using the hydrochloric acid (HCl). This is due to the fact that working with hydrochloric acid (HCl) has caused more difficulty such as dealing with violent foaming, overflowing of the contents from the digestion tubes upon the addition of the hydrogen peroxide (H_2O_2), and permanent staining on the digestion and collection tubes which also constitute as a risk by introducing these samples into the analytical instrument, as cleansing could become demanding.

Concurrently, when looking at the results for the elemental analysis, the difference in the concentration value measured between using the two types of acids is comparable. The nitric acid (HNO_3) for majority of elements and variety of mass examined, has generated much ideal values than that of using hydrochloric acid (HCl). Both the physical preparation process and result of the analysis are in favor of the nitric acid (HNO_3).

Therefore, the experiment proceeded with using nitric acid (HNO_3) for the digestion process.

4.2. Second phase: Optimization of the mass of sample and volume of acid used for digestion

The mass of the sample and volume of the nitric acid to be used for the digestion process is then investigated. 1 g, 2 g, and 3 g with 5 cm³ of nitric acid (HNO₃); 1 g, 2 g, and 3 g with 10 cm³ of nitric acid (HNO₃); and 1 g, 2 g, and 3 g with 15 cm³ of nitric acid (HNO₃) are compared from one another from the measurements made by ICP-OES and ICP-MS.

Elements detected by ICP-OES includes Al, B, Ba, Ca, Cu, Fe, K, Mg, Mo, Na, P, S, Sr, Ti, and Zn under ppm (mg kg⁻¹) range. While other elements such as As (LOD = 0.050 mg kg⁻¹), Cd (LOD = 0.050 mg kg⁻¹), Co (LOD = 0.050 mg kg⁻¹), Hg (LOD = 0.050 mg kg⁻¹), Li (LOD = 0.075 mg kg⁻¹), Ni (LOD = 0.100 mg kg⁻¹), Pb (LOD = 0.080 mg kg⁻¹), Se (LOD = 0.250 mg kg⁻¹), and V (LOD = 0.090 mg kg⁻¹) were under the limit of detection (LOD).

For analysis with ICP-MS, elements such as Li, Sc, V, Cr, Mn, Co, Ni, Cu, Se, Y, Mo, Cd, La, Nd, and Pb were detected under µg kg⁻¹ range.

A statistical analysis performed for evaluation of the optimal mass of the sample and volume of the acid for the digestion process.

For the majority of the elements measured by both ICP-OES and ICP-MS, there is a clear distinction that 1 gram of sample is best suited to be used.

The situation with the different mass of the sample used is clearly illustrated by Al in Figure 8. The significant difference between the effect of mass samples tested is evident from the graph. Where, in all cases of volumes examined, 1 g of sample produced the highest concentration value, followed by 2 g of sample and then 3 g of sample for both the 5 cm³ and 15 cm³ of nitric acid used. While for 10 cm³ it can be observed that there is no significant difference between using 2 g and 3 g of sample. In case of Al, the effect between different volume was not demonstrated properly because there is no significant difference between all 3 different volumes used: 5 cm³, 10 cm³, and 15 cm³ for both 1 g and 2 g of sample. Although, a significant difference was measured in 3 g of sample between the 5 cm³ and 15 cm³ of acid in comparison to 10 cm³ of acid used.

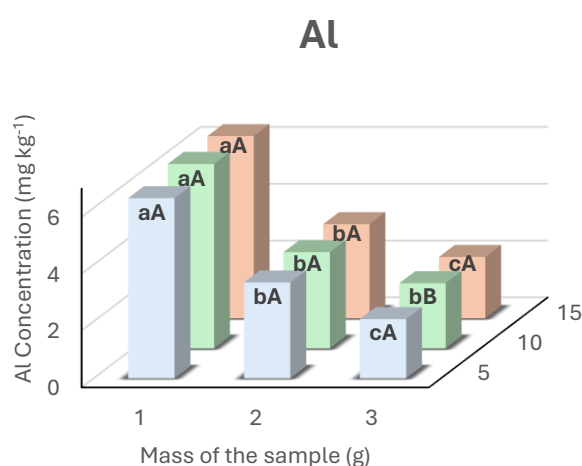


Figure 8. Concentration (mg kg⁻¹) of Al in case of optimization of mass of the sample and volume of the acid by ICP-OES. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different mass. Upper case letters signify the significant difference ($p \leq 0.05$) of the effect between different volume.

Source: own data (2024)

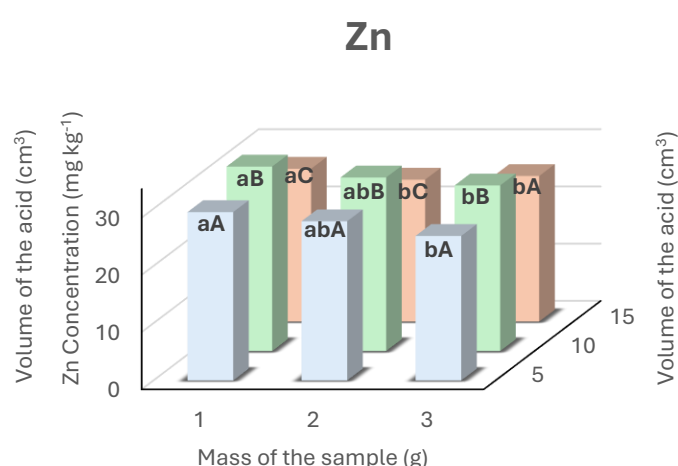


Figure 9. Concentration (mg kg⁻¹) of Zn in case of optimization of mass of the sample and volume of the acid by ICP-OES. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different mass. Upper case letters signify the significant difference ($p \leq 0.05$) of the effect between different volume.

Source: own data (2024)

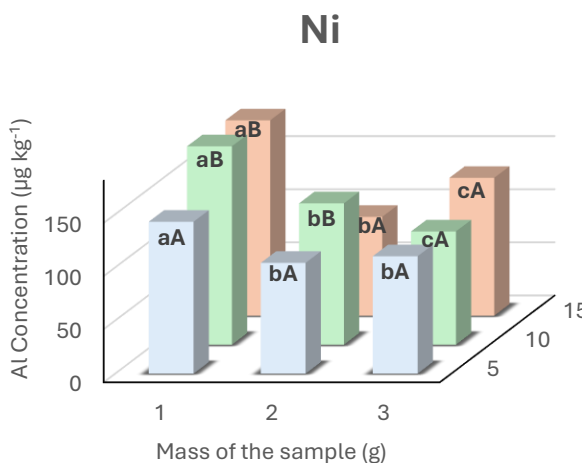


Figure 10. Concentration (µg kg⁻¹) of Ni in case of optimization of mass of the sample and volume of the acid by ICP-MS. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different mass. Upper case letters signify the significant difference ($p \leq 0.05$) of the effect between different volume.

Source: own data (2024)

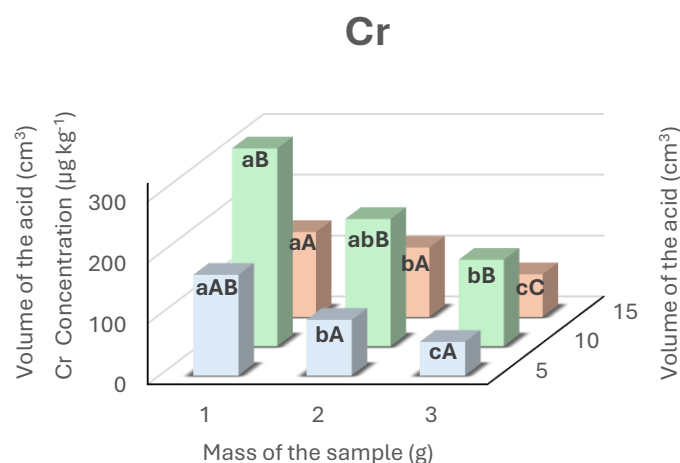


Figure 11. Concentration (µg kg⁻¹) of Cr in case of optimization of mass of the sample and volume of the acid by ICP-MS. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different mass. Upper case letters signify the significant difference ($p \leq 0.05$) of the effect between different volume.

Source: own data (2024)

The concentration of the Zn against the mass of the sample mass and volume of the acid used is shown in Figure 9. The effect of the different mass samples is exhibited properly, it can be seen that in case of 5 cm³ and 10 cm³ there is a significant difference between using 1 g and 3 g of sample, but there is no significant difference found between 1 g compared to 2 g and 2 g compared to 3 g. While in the case of 15 cm³ there is a significant difference between using 1 g of sample possessing a much higher concentration value in comparison to 2 g and 3 g of sample which is measured to have no significant difference. Regarding the effect of the different volume of the acid used, it is revealed that for 1 g and 2 g of sample there is a significant difference between using 5 cm³, 10 cm³, and 15 cm³ of nitric acid, wherein the 10 cm³ gave rise to the highest concentration value among the 3 volumes investigated. For the situation with 3 g, there is no significant difference between the 5 cm³ and 15 cm³ of nitric acid used but there is compared to using 10 cm³ of nitric acid which resulted in a much higher concentration value.

The concentration of Ni in ppb ($\mu\text{g kg}^{-1}$) was measured against the mass of sample and the volume of the acid used as illustrated in Figure 10. The effect of the different mass sample is explained properly through the results where for 5 cm³ significant difference between using 1 g of sample in contrast to 2 g and 3 g of sample which generated lower concentration values than that of 1 g. For both 10 cm³ and 15 cm³, it can be observed that there is significant difference between using 1 g, 2 g, and 3 g of sample where 1 g of sample resulted in a much higher concentration value among all mass tested. In case of the effect of the volume of the acid used, for 1 g of sample, it shows that there is a significant difference between using 5 cm³ compared to 10 cm³ and 15 cm³, where the latter produced a much higher concentration value than the prior. In 2 g of sample, there is no significant difference between using 5 cm³ and 15 cm³ of acid but there is as opposed to using 10 cm³ with a much higher concentration value. Meanwhile, there is no significant difference between all volumes (5 cm³, 10 cm³, and 15 cm³) of acid used where the 3 g of sample is concerned.

Cr was also detected and its concentration in $\mu\text{g kg}^{-1}$ was measured against the mass of sample and the volume of the acid used as illustrated in Figure 11. The difference in the effect of the variety of sample mass used is distinctly displayed. Looking at the condition when 5 cm³ and 15 cm³ of acid is applied, there is significant difference between using 1 g, 2 g, and 3 g of sample where 1 g of sample resulted in a much higher concentration value among all mass tested. For 10 cm³ of acid, there is also a significant difference between using 1 g and 3 g of sample. Here, the 1 g of sample also resulted in a much higher concentration value compared

to 3 g of sample, while the concentration value of 2 g of sample lies in between the two. The effect of the different volume of the acid used is evident even through a simple visual evaluation from the graph. It can be seen that for all cases of the sample mass inspected in the study, the 10 cm³ generated the highest concentration value among the 3 investigated volumes. Where for 1 g of sample, there is a significant difference between using 5 cm³, 10 cm³, and 15 cm³, where 10 cm³ produced a much higher concentration among all of them. Using 2 g of sample, it can be seen that there is no significant difference between using 5 cm³ and 15 cm³ of acid, but there is when compared to 10 cm³. Lastly, in the case of 3 g sample, there is a significant difference between using 5 cm³, 10 cm³, and 15 cm³. Where the highest concentration value was produced by 10 cm³, followed by 15 cm³, then 5 cm³.

According to the data measured for most elements, it is possible to state that the higher the mass of the sample utilized for the digestion process, the lower the concentration of the elements measured in the milk powder. Hence, the recommended mass of the sample to be used for the method is 1 g of skimmed milk powder.

Considering the chosen mass of the sample to be used, the volumes of the acid used is then compared to each other. It is apparent that for most elements, there is a significant difference between the volumes with the 10 cm³ having the highest concentration value among the 3 volumes tested. Although in a few cases of some elements detected, there is no significant difference between the 5 cm³ and 10 cm³ (S, Sc) or between the 10 cm³ and 15 cm³ (P, Na, Mg, Ni). On this account, it is deemed appropriate to choose 10 cm³ because it covers a much wider range of elements to be identified while producing high concentration values than that of simply 5 cm³.

The 1 g of sample with 10 cm³ of nitric acid is suitable specifically for measuring elements such as Mg, Ca, K, Fe, S, Na, P, B, Cu, Cr, Mn, Al, B, and S. It is discovered that the concentration value is slightly higher with significant difference for Li when 1 g with 15 cm³ of nitric acid is used, while for Ba when 1 g with 5 cm³ of nitric acid is used.

The compromised mass of sample and volume of the acid used to proceed with the experiment is 1 g milk powder with 10 cm³ of nitric acid, respectively.

4.3. Third phase: Optimization of the time and temperature for the pre-digestion process

The temperature and duration for the pre-digestion step was then checked for optimization. The elemental analysis was done and elements such as Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Sr, Ti, and Zn were detected while As (LOD = 0.050 mg kg⁻¹), Cd (LOD = 0.050 mg kg⁻¹), Co (LOD = 0.050 mg kg⁻¹), Cr (LOD = 0.050 mg kg⁻¹), Hg (LOD = 0.050 mg kg⁻¹), Li (LOD = 0.075 mg kg⁻¹), Ni (LOD = 0.100 mg kg⁻¹), Pb (LOD = 0.080 mg kg⁻¹), Se (LOD = 0.250 mg kg⁻¹), and V (LOD = 0.090 mg kg⁻¹) were under the limit of detection (LOD).

A statistical analysis was carried out to determine the best temperature and duration to be used for the pre-digestion.

The concentration of P was plotted against time and temperature as shown in Figure 12. It can be observed that, in case of 30°C there is no significant difference between the concentration values measured with 30 min and 60 min but there is a significant difference when these values are compared to 90 min. At 60°C, there is no significant difference between the concentration values between all the duration time for pre-digestion. While in case of 90°C, the concentration value is between 60 min and 90 min for the pre-digestion made at 30 min duration, with 60 min having a smaller concentration value compared to 90 min duration. On the other hand, upper case letters signify the significant difference of the effect between different temperature. It can be seen that, for both 30 min and 60 min there is no significant difference between all 3 temperatures. However, for the 90 min duration, there is a clear significant difference between all 3 temperatures, with 30°C possessing the least concentration value among the three, followed by 60°C, and 90°C being the highest.

The Ca concentration was plotted against the examined parameters as demonstrated in Figure 13. For the effects of the different time, it can be observed that, in case of 30°C and 90°C there is no significant difference between the concentration values measured at all 3 of the time duration for the pre-digestion. But in case of the 60°C, there is a significant difference between the concentration values measured at 30 min compared to 60 min and 90 min which shows no significant difference from each other. Regarding the effect of the different temperature, it shows that for both 30 min and 60 min, there is no significant difference between concentration values measured at both 30°C and 60°C in comparison to the values measured at 90°C. Meanwhile, similarly to the P, in the 90 min pre-digestion there is a clear significant difference

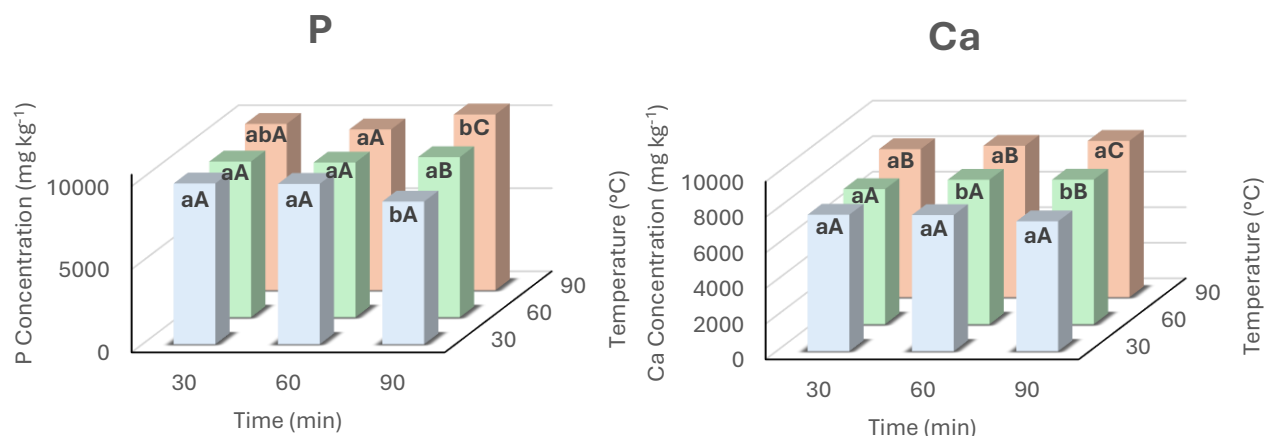


Figure 12. Concentration (mg kg^{-1}) of P in case of optimization of different temperature and duration of the pre-digestion step. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different time. Upper case letters signify the significant difference ($p \leq 0.05$) of the effect between different temperature.

Source: own data (2024)

Source: own data (2024)

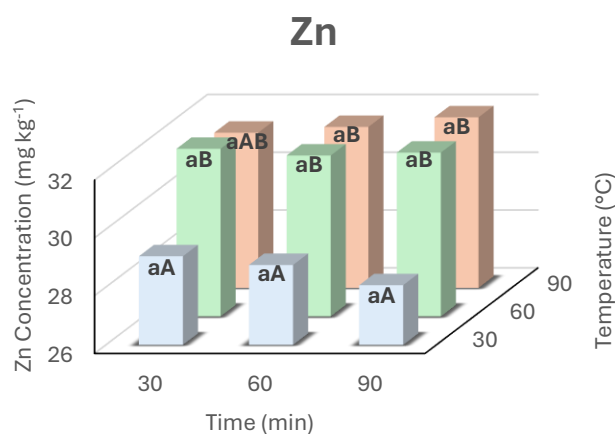


Figure 14. Concentration (mg kg^{-1}) of Zn in case of optimization of different temperature and duration of the pre-digestion step. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different time. Upper case letters signify the significant difference ($p \leq 0.05$) of the effect between different temperature.

Source: own data (2024)

among all 3 temperatures, with 30°C possessing the least concentration value, followed by 60°C, and 90°C being the highest.

In case of Zn, the concentration was also plotted against the examined parameters as displayed in Figure 14. It is evident that there is no significant difference among all 3 durations for all 3 temperature case, implying that there is hardly any effect upon varying the duration of the pre-digestion step in case of Zn. Although, for the effect of the different temperatures, in almost all case of duration, it is clear that there is a significant difference between 30°C having much lower concentration values at all 3 durations (30 min, 60 min, and 90 min) compared to 60°C and 90°C with the exception of 30 min at 90°C being between the two values.

Based on the results, the circumstance varies as some elements show visible significant difference from when the temperature are varied while the others on the duration. The 90 min duration and 90°C is especially fitting for measuring elements such as S, P, Ca, Cu Mg, and Mn because they exhibit a calculated significant difference and produced the highest concentration value measured at this specific condition. Regarding Zn, it can be assured that 30°C is not suitable for the analysis as it shows that there is a significant difference and too low of a concentration value measured at this temperature compared to when either 60°C or 90°C was used.

Therefore, the compromised time and duration recommended for the pre-digestion step is 90 min and 90°C.

4.4. Fourth phase: Optimization of the volume of the hydrogen peroxide (H₂O₂) added

The volume of the cc. hydrogen peroxide (H₂O₂) 1 cm³, 3 cm³, and 5 cm³ added is then examined for optimization. The elemental analysis was done and elements such as Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Sr, Ti, and Zn were detected under ppm (mg kg⁻¹) range, while elements such as As (LOD = 0.050 mg kg⁻¹), Hg (LOD = 0.075 mg kg⁻¹), Li (LOD = 0.075 mg kg⁻¹), Ni (LOD = 0.100 mg kg⁻¹), Pb (LOD = 0.080 mg kg⁻¹), Se (LOD = 0.250 mg kg⁻¹), and V (LOD = 0.090 mg kg⁻¹) were under the limit of detection.

A statistical analysis was carried out to determine the optimal volume of the hydrogen peroxide (H₂O₂) to be added for the digestion. For most elements, the concentration values have no significant difference between the different volume that was used. This is illustrated similarly to the situation of Al as shown in Figure 15. It is also readily apparent from the figure that the concentration values from the 3 parameters (1 cm³, 3 cm³, and 5 cm³ of H₂O₂) tested were estimated the same.

For several elements like in the case of P which is demonstrated in Figure 16, it can be observed that there is a significant difference between using 1 cm³ compared to using 3 cm³ and 5 cm³ which possess no significant difference from each other. The concentration value when using 3 cm³ and 5 cm³ of H₂O₂ for the digestion generates much higher results, 10553 mg kg⁻¹ and 10760 mg kg⁻¹ respectively, compared to 1 cm³ of H₂O₂ having 10009 mg kg⁻¹. The same tendencies were found in: S, K, Mo, and Cr.

One case would be like that of Cu as illustrated in Figure 17. It is evident that there is no significant difference between using 1 cm³ and 3 cm³ of H₂O₂ for the digestion process. But in comparison to using 5 cm³ of H₂O₂, a significant difference can be observed, with a much higher concentration value of 1.36 mg kg⁻¹ than the other 2 lesser volumes.

Majority of the cases were either of no significant difference or identical to that of the situation as observed in P, where using 1 cm³ resulted to a much lower concentration value in comparison to that of 3 cm³ and 5 cm³. Furthermore, foaming was observed upon the addition of 5 cm³ of H₂O₂ which can be of safety concern. Considering that a wider range of elements are better detected at higher volumes than 1 cm³, and that there is no significant difference between 3 cm³ and 5 cm³, it is therefore recommended to use 3 cm³ of H₂O₂ to be added for the digestion process.

Al

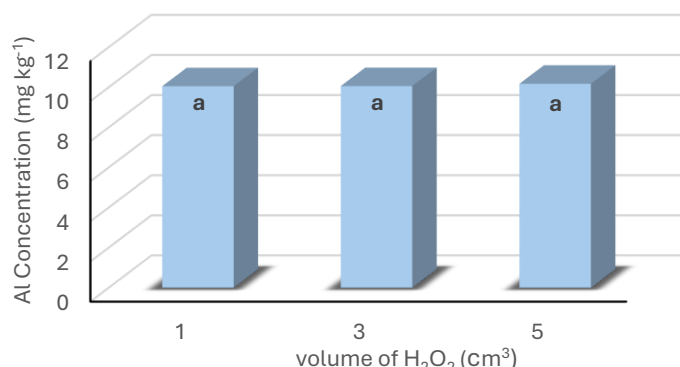


Figure 15. Concentration (mg kg⁻¹) of Al in case of optimization of different volume of H₂O₂ added. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different volume of H₂O₂

Source: own data (2024)

P

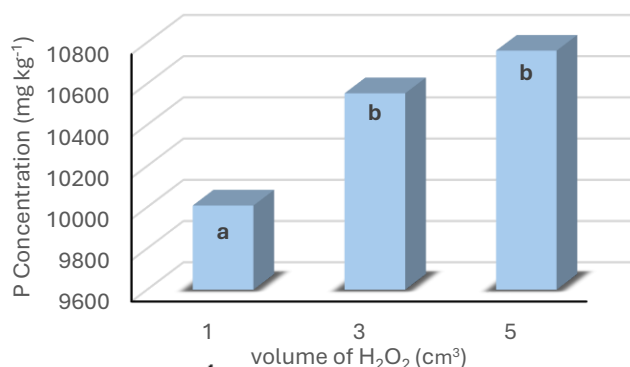


Figure 16. Concentration (mg kg⁻¹) of P in case of optimization of different volume of H₂O₂ added. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different volume of H₂O₂

Source: own data (2024)

Cu

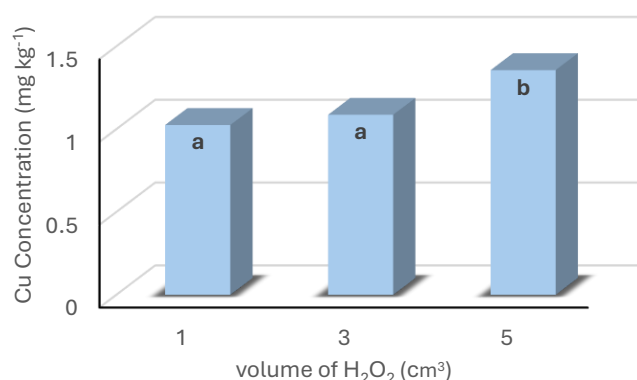


Figure 17. Concentration (mg kg⁻¹) of Cu in case of optimization of different volume of H₂O₂ added. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different volume of H₂O₂

Source: own data (2024)

4.5. Final phase: Optimization of the time and temperature for the main digestion process

For the final step, the time and temperature for the main digestion process was investigated for optimization. The elemental analysis was done and elements such as Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Sr, Ti, and Zn were detected while other were under the limit of detection (LOD) including As (LOD = 0.050 mg kg⁻¹), Cd (LOD = 0.050 mg kg⁻¹), Co (LOD = 0.050 mg kg⁻¹), Cr (LOD = 0.050 mg kg⁻¹), Hg (LOD = 0.050 mg kg⁻¹), Li (LOD = 0.075 mg kg⁻¹), Ni (LOD = 0.100 mg kg⁻¹), Pb (LOD = 0.100 mg kg⁻¹), Se (LOD = 0.250 mg kg⁻¹), and V (LOD = 0.090 mg kg⁻¹).

A statistical analysis was carried out on the measurement result to determine the optimal time and duration for the main digestion. The concentration of K was plotted against time and temperature as shown in Figure 18. The effect of the time can be observed. In case of 100°C, there is a significant difference between the effect of the time where at 60 min the concentration value is the lowest and the highest at 120 min, while the concentration value of the main digestion at 90 min is amidst the two. For main digestion at 120°C, it is evident that there is a significant difference between using 60 min duration for the main digestion in comparison to using 90 min and 120 min. The concentration value is much lower from the 60 min, but much higher with 90 min and 120 min wherein there is no significant difference between the values of the latter two. At 140°C, it can be stated that there is no significant difference between the effects of different duration times. The effects of the different temperature can be detected in the 60 min duration where, there is a significant difference between using 100°C generating the lowest concentration value, 140°C being the highest, with 120°C being between the two values. For both the 90 min and 120 min, it can be seen that there is no significant difference between the different temperatures used for the main digestion step (100°C, 120°C, and 140°C).

The situation involving Ca is demonstrated in Figure 19. The figure shows that for 100°C, there is a significant difference between using 60 min duration in comparison to using 90 min and 120 min duration where the latter two time duration poses no significant difference from each other while also having a much higher concentration value measured than from the 60 min duration. For both 120°C and 140°C, there is a significant difference between the 60 min duration and 120 min duration while the 90 min duration falls in between both the values. When inspecting the effect of the main digestion temperature, in case of the 60 min and 120 min

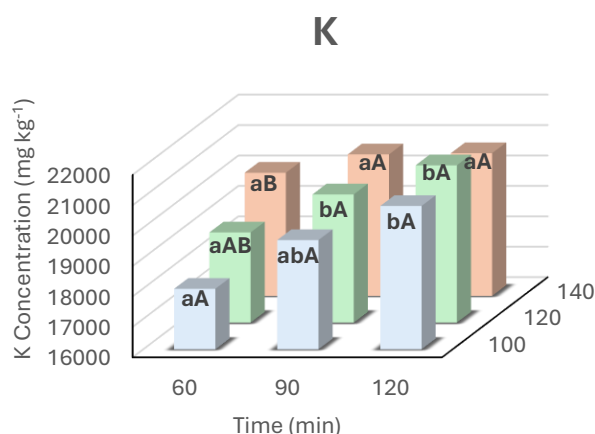


Figure 18. Concentration (mg kg⁻¹) of K in case of optimization of different temperature and duration of the main digestion step. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different time. Upper case letters signify the significant difference ($p \leq 0.05$) of the effect between different temperature.

Source: own data (2024)

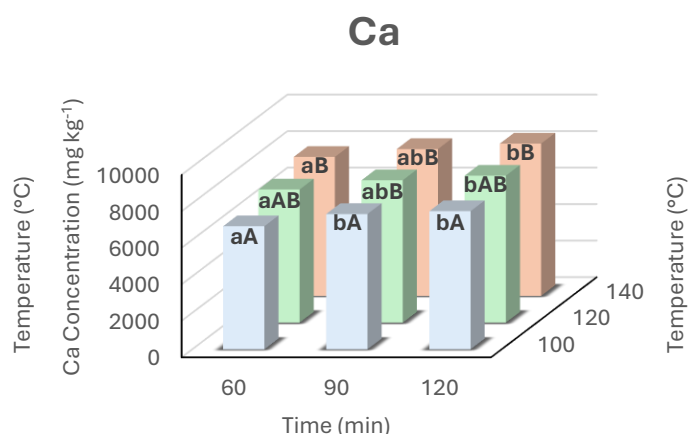


Figure 19. Concentration (mg kg⁻¹) of Ca in case of optimization of different temperature and duration of the main digestion step. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different time. Upper case letters signify the significant difference ($p \leq 0.05$) of the effect between different temperature.

Source: own data (2024)

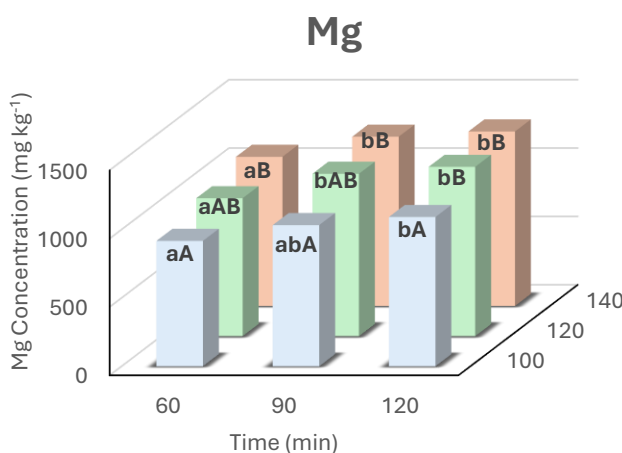


Figure 20. Concentration (mg kg⁻¹) of Mg in case of optimization of different temperature and duration of the main digestion step. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different time. Upper case letters signify the significant difference ($p \leq 0.05$) of the effect between different temperature.

Source: own data (2024)

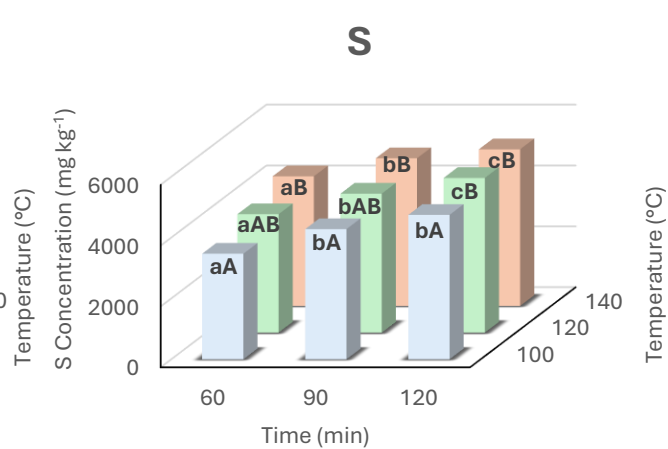


Figure 21. Concentration (mg kg⁻¹) of S in case of optimization of different temperature and duration of the main digestion step. Lower case letters signify the significant difference ($p \leq 0.05$) of the effect between different time. Upper case letters signify the significant difference ($p \leq 0.05$) of the effect between different temperature.

Source: own data (2024)

duration 100°C generated the lowest concentration value, 140°C being the highest, with 120°C being between the two values. While with using 90 min duration, the significant difference is apparent between using 100°C when compared to 120°C and 140°C. It is clear that the concentration value in the case of 100°C is much lower than 120°C and 140°C wherein there is no significant difference found between the two according to statistical computation.

The concentration of Mg was also plotted against the temperature and time duration as presented in Figure 20. The behavior of the result from the statistical analysis is similar to that of Ca. In case of 100°C, there is a significant difference between the effect of the time where at 60 min the concentration value is the lowest and the highest at 120 min, while the concentration value of the main digestion at 90 min is amidst the two. On the other hand, digestion made at 120°C and 140°C both show that there is a significant difference between the concentration value measured at 60 min duration possessing a much lower value compared to the result measured at 90 min and 120 min which are much higher and shows no significant difference from each other. Considering the effect of the temperature, it is also clearly shown that in the case of the 60 min and 90 min duration, there is a significant difference between digestion made at 100°C which generated much lower concentration value compared to 140°C, while digestion made at 120°C fall amidst both values. Digestion at 120 min duration also shown significant difference between the temperature of 100°C having a much lower concentration values measured than that of both 120°C and 140°C which were calculated to have no significant difference from one another.

The circumstance in regard to S is slightly different as illustrated in Figure 21. It can be observed that except for the digestion made at 100°C where there is only a significant difference between the 60 min duration compared to 90 min and 120 min which have no significant difference from each other, for other cases (120°C and 140°C), there is a distinct significant difference between all three time duration investigated (60 min, 90 min, and 120 min). Where the lowest concentration values were produced by digestion at 60 min, followed by 90 min, then the highest by the 120 min duration. Evaluating the effect of the temperature, it can be expressed that for both 60 min and 90 min duration, there is a significant difference between using 100°C which generated the lowest concentration value, 140°C being the highest, with 120°C being between the two values. While in the case of the digestion made at 120 min duration, it can be seen that there is a significant difference between using 100°C with measured

value much lower than using 120°C and 140°C which were calculated to have no significant difference from each other.

Based on the findings, in terms of the effect of the time duration used for the main digestion process, it can be presumed that 60 min is not ideal due to exhibiting a much lower concentration value measured with a significant difference from that of measured at 90 min and 120 min duration for most elements. Since there is no significant difference between the values measured at 90 min and 120 min duration, it is more befitting to use 90 min due to less time and energy input in the process.

The effect of the temperature is more pronounced and consistent for most elements measured in this phase. As demonstrated in the figures, using 100°C generates a much lower concentration value with a significant difference compared to digestion made at 120°C and 140°C. Considering that there is no significant difference between 120°C and 140°C, it is unnecessary to use a much higher temperature only to achieve the same result as that of a much lower temperature, and therefore it is more beneficial to use 120°C in terms of efficiency and effectiveness.

For most elements measured, it is more frequent to find that there is no significant difference found between the time duration of 90 min and 120 min, and between the temperature of 120°C and 140°C. The 120°C and 90 min duration condition is particularly beneficial for the analysis of distinct elements such as Ca, K, Mg, Mn, and P. In case of S, it can be observed that 120°C and 120 min is the most ideal case due to a significant difference between all the time duration examined with 120 min being the highest while there is no significant difference between 120°C and 140°C.

Thus, the compromised temperature and time duration recommended for the main digestion step is 120°C and 90 min, respectively.

5. CONCLUSIONS AND RECOMMENDATIONS

As the movement of milk powder is more beneficial in terms of bulk transport and cost, while simultaneously lowering the risk of spoilage and therefore waste, in an incident where improper handling could take place, it is still essential that a proper analysis of the milk powder must be made to ensure the quality and establish safety of the product to prevent any hazards from causing detrimental effects to the public's health.

Some matters to consider include the exposure of the product to different environment throughout its movement, as well as the product contact materials whether it is during the production, shipping, transport, receiving, storage or before further processing is intended, where contamination of toxic and/or heavy metals can possibly occur should be monitored and emphasized. The proposed parameters are recommended for an open-system wet digestion sample preparation method of milk powder for the elucidation of its elemental content.

In this study, a quantitative and statistical analysis was made for the majority of the conditions to assess the values chosen for each parameter which was decided through examining the concentration values generated as the condition was set in place when the experiment was carried out.

Nevertheless, both a qualitative and quantitative analysis was involved for the selection of the type of acid to be used in the initial step. The statistical comparison for the rest of the parameters was made to aid the evaluation, to confirm and increase confidence in the data for the decision of the proposed value.

The advantage of the method includes a much cheaper equipment as well as higher number of samples can be loaded for the digestion process at once in contrast to microwave digestion techniques.

An open-system wet digestion method was applied for the determination of the optimal parameters for the sample preparation process of the skimmed milk powder.

The results from each phase of the study were meticulously analysed, calculated, compared, and evaluated in order to decide the most suitable conditions for the elemental analysis of skimmed milk powder. Table 6 summarizes the parameters examined and outcome of the investigation for the compromised optimal parameters of the digestion method described.

Table 6. The parameters studied and result for the compromised optimal condition for the digestion method

Category	Value
Type of Acid	HNO ₃
Mass of sample	1 g
Volume of HNO ₃	10 cm ³
Temperature for pre-digestion	90°C
Duration of pre-digestion	90 min
Volume of H ₂ O ₂	3 cm ³
Temperature for main digestion	120°C
Duration of main digestion	90 min

Source: own data (2024)

It can be observed that nitric acid (HNO₃) was the most ideal type to be utilized for the digestion of the skimmed milk powder compared to hydrochloric acid (HCl) due to both qualitative performance and quantitative results from the elemental analysis data which were evaluated for comparison.

The mass of the sample to be used was the easiest to determine due to significant differences in most elements measured with it possessing the highest concentration values compared to other mass used.

The compromised time and temperature for the pre-digestion step were 90°C and 90 min.

The optimal volume of the hydrogen peroxide (H₂O₂) was found to be 3 cm³, because although there is no significant difference between the effect of volumes for several elements, some concentration values were found the highest at 3 cm³ or between 3 cm³ and 5 cm³. But due to occurrence of foaming when 5 cm³ was added, it was ascertained that using 3 cm³ of the acid was environmentally and economically more beneficial.

Through comparison and statistical evaluation, that compromised time and temperature for the main digestion process were determined to be 120°C and 90 min, respectively.

The application of the study covers the analysis of the elemental components of milk powder, concerning possible toxic or heavy metal contamination, which stands as a threat for consumer safety. The method can also be of significance for assuring and inspecting milk powder products with claims of mineral fortification.

For further development of the method, it would be beneficial to investigate more than one type of sample, such as products from other brands or various fat content of milk powders, to assess the validity and applicability of the method. The type of acid to be examined for the digestion could also be diversified, to check any significant differences on the analysis.

The parameters of the analytical instrument used could also potentially be a topic of investigation, such as the determination of the intersection of the optimal parameters of the sample preparation method and the inductively coupled plasma spectrometer analysis.

SUMMARY

The study presents a method to achieve efficient and effective elemental analysis of milk powder, to address concerns in terms public safety and quality of the product's potential presence of toxic and heavy metals. A proper analysis of the milk powder can provide reliable information regarding elemental content of the sample, wherein the sample preparation process plays a crucial role. Developing a method with optimal conditions can aid a proper analysis to be carried out.

In order to find the optimal conditions for the method, each parameter was investigated to determine the best option for the analysis. The initial step involved determining the type of acid best suited for the digestion of the skimmed milk powder sample under the same conditions. Once the type of acid was determined, the mass of the sample and volume of the acid was then examined and visually compared for any difference or changes. When the mass of the sample and the volume of the acid was set, the time and temperature for the pre-digestion is then varied for optimal condition. After the pre-digestion, hydrogen peroxide H_2O_2 is usually added. The volume of the peroxide was then inspected to decide how much amount of the hydrogen peroxide H_2O_2 would be ideally added to the sample solution. Once the volume of the sample was determined, the final phase, which is the parameters of the main digestion, was then experimented on. The time and temperature of the main digestion was varied for choices as to which conditions it would be best carried out.

The instruments used for the analysis of the elements includes Thermo Scientific iCAP 6300 Dual view inductively coupled plasma optical emission spectrometry (ICP-OES) and Thermo Scientific X-Series II inductively coupled plasma mass spectrometry (ICP-MS) which was used for comparison for the determination of mass of the sample and volume and type of acid to be used.

Different evaluation method was implemented to analyze the results obtained from the elemental analysis. IBM SPSS Statistics V22.0 was used for the statistical analysis, where one-way ANOVA and Tukey-test were applied to examine the relationships between parameters and factors. Differences were considered significant at $p \leq 0.05$.

Based on the results, in case of the type of acid for the digestion, through both visual and analytical evaluation, it can be declared that nitric acid (HNO_3) is most suited to be used compared to hydrochloric acid (HCl) due to both better concentration values obtained from the

analysis, as well as convenience of use by not causing foaming, spilling, and permanent staining on the tubes as samples with hydrochloric acid (HCl) did.

The concentration values detected at each mass of the sample as well as volume of the acid used was statistically compared to each other, and the results state that the optimal mass of sample to be used was 1 g while volume being 10 cm³.

For the parameters of the pre-digestion process, it was found that the compromised optimal condition to be used is where the temperature is at 90°C and the duration being 90 min.

The result of the analysis for the volume of the hydrogen peroxide (H₂O₂) added had almost no significant difference from each other but taking into consideration that a wider range of elements are better detected at higher volumes than 1 cm³, and that there is no significant difference between 3 cm³ and 5 cm³, where foaming also occurred upon the addition of 5 cm³ of H₂O₂ which can be of safety and quality concern, 3 cm³ of (H₂O₂) was therefore chosen as the most suitable volume to be added.

For the main digestion process, it was observed that for most elements measured, it is more frequent to find that there is no significant difference found between the time duration of 90 min and 120 min, and between the temperature of 120°C and 140°C. For certain elements such as Ca, K, Mg, Mn, and P, the concentration values were found to be in favor of 120°C and 90 min duration. Except for S where 120°C and 120 min is the most ideal case due to a significant difference between all the time duration examined with 120 min being the highest while there is no significant difference between 120°C and 140°C. Hence, the compromised temperature and time duration recommended for the main digestion step is 120°C and 90 min, respectively.

The compromised optimal condition for the elemental analysis of milk powder is then concluded as using 1 g of skimmed milk powder with 10 cm³ of nitric acid (HNO₃) that is pre-digested at 90°C for 90 min. After cooling down the samples to room temperature, 3 cm³ of H₂O₂ shall be added and placed back into the digestion block for main digestion at 120°C for 90 min.

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DECLARATION

I, the undersigned.....DIANE OLIVAREZ BARTOLOME....., a student of the Faculty of Agricultural and Food Sciences and Environmental Management of Debrecen University, being fully aware of my legal liability, do hereby declare and certify with my signature that my TDK work is the result of my own original work. I handled the used literature in a correct way, I learned the way of correct handling of literary sources from the regulations, I complied with the legislation on the diploma thesis. I acknowledge that in the event of a breach of these requirements, my thesis will be rejected.

Diane Olivarez Bartolome

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signature

Date of birth:.....29th of May 2001.....