

Methods and Protocols
in Food Science

Springer Protocols

José Manuel Lorenzo · Rubén Domínguez
Mirian Pateiro · Paulo E. S. Munekata *Editors*

Methods to Assess the Quality of Meat Products

 Humana Press

METHODS AND PROTOCOLS IN FOOD SCIENCE

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Methods to Assess the Quality of Meat Products

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ISSN 2662-950X ISSN 2662-9518 (electronic)
Methods and Protocols in Food Science
ISBN 978-1-0716-2001-4 ISBN 978-1-0716-2002-1 (eBook)
<https://doi.org/10.1007/978-1-0716-2002-1>

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The registered company address is: 1 New York Plaza, New York, NY 10004, U.S.A.

Preface

The meat industry is a sector with great importance worldwide. However, current consumer trends as well as regulatory changes make essential the determination of the constituents and the additives. The different analytical techniques that can be applied arise from the need to provide correct nutritional information in accordance with the legislation (labeling), as well as to determine the nutritional quality and chemical composition of meat and meat products. On the other hand, the physicochemical and degradative changes that can take place can also be a source of significant economic losses or even lead to the appearance of toxic substances, which would put the health of the consumer at risk. Therefore, the determination of these changes should be also carried out.

This book arises from the collaboration between different institutions and centers at an international level, which are members of the Healthy Meat network, funded by CYTED (ref. 119RT0568). Therefore, the publication of this book is a very useful tool in order to standardize the analytical processes, as well as to serve as a basis for the development and validation of these techniques in those research groups that do not have them implemented.

Several analytical techniques have been developed for each of the determinations described in this book. However, the variations between them as well as the use of different techniques could lead to the results obtained not being reproducible or comparable. With this in mind, the current book examines the techniques used for the analysis of meat and meat products. A complete and comprehensive description of the methods and materials used is made so that each technique can be reproduced by other researchers. In addition, the techniques include explanatory notes and elucidate the possible points to take into account for the correct achievement of the determination. The ultimate goal is to support the scientific community, professionals, laboratories, food companies, and regulatory bodies in their aim to identify and measure meat and meat products composition and properties. The book consists of 14 chapters.

Chapter 1 provides the complete material and methods description for the determination of the different chemical parameters, including moisture, fat, protein, and ash. In addition to being mandatory to include part of this information on the products labeling, these determinations also offer nutritional information on the final product.

Chapter 2 discusses the techniques used for the measurement of pH and color. In this case, color is considered one of the most important parameters that influence consumer acceptance. Thus, the correct determination of physical (CIELab) and also chemical (haem pigments) color is important to control the meat and meat products quality.

Not only color but also texture is important in consumer preferences. With this in mind, Chapter 3 provides different procedures for the correct determination of texture parameters in different meat and meat products.

Chapter 4 includes the complete method for fat extraction, transesterification, and determination of fatty acids with the aim to have complete nutritional information. This is important to quantify saturated fatty acids (necessary for products labeling) and also for nutritional claims (e.g., source of omega-3, low saturated fats, etc.). Additionally, the fatty acid determination also provides information about the lipid stability of the product.

In Chapter 5, a complete description of free amino acids and hydrolyzed amino acids extraction is explained, followed by the derivatization and chromatographic analysis. The

free amino acids content is related to the proteolytic phenomena during meat products manufacturing and processing, while hydrolyzed amino acids indicate the nutritional quality of meat and meat products (e.g., essential amino acids).

Chapter 6 deals with the analysis of total cholesterol in meats. It is well known that cholesterol consumption is related to the development of some human diseases. Thus, the correct determination of this compound provides both the consumer and the manufacturer with important information.

Similarly, the content of several minerals is important to human health. Thus, in Chapter 7, a comprehensive explanation about the sample treatment and mineral composition analysis using IPC-OES is provided.

Chapter 8 focuses on the analytical determination of nitrites and nitrates in meat and meat products, using liquid chromatography. These additives are necessary for several meat products manufacture since they control the growth of some pathogenic microorganisms and stabilize the color. However, they are also related to the development of toxic molecules; thus the residual content of both is regulated.

The release of biogenic amines during processing, mostly in fermented or ripened products, is also frequent. These compounds are related to some allergenic reactions and are harmful for humans. Therefore, Chapter 9 provides a complete description of the materials and methods for their determination using liquid chromatography.

On the other hand, it is well known that oxidative processes are the most important degradative reactions during both the processing and storage of meat and meat products. They produce loss of nutrients, reduce sensory properties, develop dangerous compounds, and decrease their shelf life. With this in mind, the analysis of protein carbonyls using a spectrophotometric technique for the determination of protein oxidation is completely explained in Chapter 10, while comprehensive procedures for the determination of primary and secondary lipid oxidation products are described in Chapter 11.

Volatile compounds are vital for the correct development of the typical aroma of meat and meat products. Additionally, the release of some specific compounds allows the monitoring of degradative processes. Therefore, the analysis of these compounds is important to monitor the sensory quality and the manufacturing process. Chapter 12 provides a complete description of the analysis of volatile compounds using a solvent-free technique, based on solid-phase microextraction with gas chromatography-mass spectrometry determination.

Chapter 13 provides a brief overview of specific issues in the meat industry, which may be resolved using the current proteomics-based methodology. The quantification, analysis, and curation of proteomics data are explained in this chapter.

Finally, the antioxidant activity of meat and meat products could exert a protective effect against oxidative degradation. Therefore, Chapter 14 explains in a comprehensive way the main techniques used for the determination of antioxidant capacity in meat and meat products.

Ourense, Spain

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Series Preface

The *Methods and Protocols in Food Science* series is devoted to the publication of research protocols and methodologies in all fields of food science. The series is unique as it includes protocols developed, validated, and used by food and related scientists as well as theoretical bases are provided for each protocol. Aspects related to improvements, adaptations, and further developments in the protocols may also be approached.

The *Methods and Protocols in Food Science* series aims to bring the most recent developments in research protocols in the field as well as very well-established methods. As such the series targets undergraduate, graduate, and researchers in the field of food science and correlated areas. The protocols documented in the series will be highly useful for scientific inquiries in the field of food sciences, presented in such a way that the reader will be able to reproduce the experiments in a step-by-step style.

Each protocol will be characterized by a brief introductory section, followed by a short aims section, in which the precise purpose of the protocol is clarified. Then, an in-depth list of materials and reagents required for employing the protocol is presented, followed by comprehensive and step-by-step procedures on how to perform that experiment. The next section brings the dos and don'ts when carrying out the protocol, followed by the main pitfalls faced and how to troubleshoot them. Finally, template results will be presented and their meaning/conclusions addressed.

The *Methods and Protocols in Food Science* series will fill an important gap, addressing a common complaint of food scientists, regarding the difficulties in repeating experiments detailed in scientific papers. With this, the series has a potential to become a reference material in food science laboratories of research centers and universities throughout the world.

Campinas, São Paulo, Brazil

Anderson S. Sant'Ana

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Chapter 1

Chemical Composition

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Abstract

Meat composition influences the nutritional and sensory quality of meat products, therefore elucidating its composition is essential for the production chain. Moisture, fat, protein, and ash are the main components of these products whose determinations have been widely studied for many years. That is why today traditional methods, new techniques, and a host of smart equipment can be used, which could affect the results obtained and their comparison.

This chapter give a complete analysis guideline of the aforementioned determinations. Moisture determination is carried out by thermogravimetry, according to sample weight loss. Fat and protein contents are determined by Ankom technology and Kjeldahl method, respectively. Ashes are calculated by weight loss once the sample has been calcined in a muffle furnace.

Key words Moisture, Protein, Fat, Ash, Proximate composition, Gravimetric method, Ankom extractor, Hydrolysis, Kjeldahl method, Calcination process

1 Introduction

Chemical composition, also known as proximate composition, is very important in the food industry for the development of new products, quality control, consumer information or regulatory purposes. In this way, it is necessary to know the percentage of moisture, protein, fat, and ash of the products to establish rules that regulate the diet of the people to make it healthier. Therefore, these analyses are very important since they are directly related to the health of consumers, as well as for the food production [1, 2]. All of these parameters could be modified both by changing the diet of the animals or by using new ingredients during processing [3]. The best level of each of them depends on the type of consumer.

Taking into account the aforementioned facts, people have the right to know the nutritional composition of the meat and meat

products that they eat. Therefore, Governments ensure this to their citizens through several rules, such as Regulation (EU) 1169/2011 in the European Union (EU) [4] or CFR (21CFR101) of the Food and Drug Administration (FDA) of the United States of America (USA) [5], among others. So, companies have to label their products according to these regulations. They are many similarities between them, only with slight differences. According to Regulation 1169/2011, it is compulsory to label the percentage of fat, protein, carbohydrates, total sugars, sodium chloride, saturated fatty acids and energy (Kcal and KJ), as well as, all allergens presented in products. In addition, other nutrients can also be labeled, such as dietary fiber, mono and polyunsaturated fatty acids, vitamins, etc. In addition, it is necessary to include on the label important information about the origin, expiration date, etc.

All methods, which we are going to describe below, are supported by international organizations such as the International Organization for Standardization (ISO) or the American Oil Chemists Society (AOCS). Firstly, water occurs as the main constituent of meat and meat products. Therefore, the determination of the water content of meat and meat products is very important, since water is an essential constituent and its interaction with other components condition the shelf life and the quality of the product [6]. Moisture analysis is a gravimetric method based on loss of weight by drying. This method has been used in the same way for many years. Similarly, ashes determination is based on a loss of weight due to the calcination of organic matter in a muffle furnace. Secondly, lipids play an important role in sensory quality of meat and meat products, since it contributes to attributes such as texture (juiciness), the formation of the characteristic flavor of the product, and its final appearance. Therefore, fat determination is one of the required analyses in food labeling and quality control [7]. Fat analysis can be performed with or without hydrolysis, depending on the type of the sample. In many samples, fat is not freely accessible to the solvent and cannot be fully extracted. The bonding of fats in starch-lipid complexes or with lipoproteins or phospholipids, prevents direct extraction of the fat. For this reason, hydrolysis with HCl to prepare samples for fat determination is prescribed by law in a wide range of national and international methods. Fat determination with hydrolysis is known as “Total fat determination,” while the method without hydrolysis determines crude fat. In addition, the analysis of fat has changed over the years, specially, regarding the equipment used. In this regard, Soxhlet extractor sometimes has limitations, especially when the fat content is very low, which makes it not very reproducible. Below, an alternative to this apparatus is shown.

Proteins are also very important constituents of meat and meat products, since they have a high nutritional value and their structural and physicochemical characteristics are related to their

functional properties [7]. As with fat determination, protein analysis has also changed over the years regarding the equipment used, so numerous methods have been developed. Two steps are necessary, an acid digestion and a titration to obtain the total nitrogen content present in the sample. The application of a correction factor, which is specific for each type of sample, allows to calculate the percentage of protein.

The aim of this chapter is to make a guide for the analysis of the chemical composition of meat and meat products, so that they can compare the results minimizing the error due to the method used.

2 Materials

Prepare all solutions using distilled water and/or analytical grade reagents. Prepare and store all reagents at room temperature (unless indicated otherwise). Diligently follow all waste disposal regulations when disposing of waste materials (*see Note 1*).

2.1 Sample Preparation for Analysis

1. Professional mill KN 295 Knifetec (Foss).
2. Homogeniser HM 294 (Foss).
3. Cutting board.
4. Knife.

2.2 Moisture Determination

1. Drying oven with capacity to dry the samples at 105 ± 5 ° C, mod. UFP 600 (Mettler).
2. Analytical balance, with a resolution of 0.0001 g, mod. ME 614S (Sartorius).
3. Desiccator equipped with an effective dehydrator (Silica Gel with humidity indicator).
4. Stainless steel capsules of 60 mm diameter and 25 mm high.
5. Glass rod with rounded tip, which goes completely into the capsule.
6. Eppendorf Research Micropipette 500–5000 μ L.
7. Washed sea sand, fine grain. General purpose grade.
8. Ethanol 96% for analysis, ACS, Reagent grade.

2.3 Fat Determination

1. Filter Bags, mod. XT4 (Ankom).
2. Indelible marker.
3. Filters support (Ankom).
4. Stainless steel capsules of 60 mm diameter and 25 mm high.
5. Analytical balance, with a resolution of 0.0001 g, mod. ME 614S (Sartorius).

6. Thermosealer Impulse Sealer, mod. AIE-200.
7. Drying oven with capacity to dry the samples at 105 ± 5 ° C, mod. UFP 600 (Mettler).
8. Desiccator equipped with an effective dehydrator (Silica Gel with humidity indicator).
9. Extraction Unit, mod. XT10 (Ankom).
10. Ankom hydrolysis unit, mod. HCl (Ankom).
11. Weight to dry the samples after hydrolysis (Blotter), mod. H35 (Ankom).
12. Rapid Dryer HCl Filter RD2 (Ankom).
13. Support for samples.
14. Borosilicate glass graduated cylinder of 250 mL, class A.
15. Solvent recovery glass bottle 500 mL.
16. Petroleum ether with a boiling point range 40–60 °C, reagent grade, ACS, ISO (*see Note 2*).
17. Celite Hyflo Super Cel, RE.
18. Hydrochloric acid solution (3 N): Mix 248 mL of hydrochloric acid (37%) with 752 mL of distilled water.

2.4 Protein Determination

1. Analytical balance, with a resolution of 0.0001 g, mod. ME 614S (Sartorius).
2. Precision balance with a resolution of 0.01 g, mod. TE 612 (Sartorius).
3. Temperature Controller, mod. TZ (Gerhardt).
4. Nitrogen Digester Kjeldatherm, mod. KB20 (Gerhardt).
5. Nitrogen Distiller, mod. VAPODEST 50 carousel (Gerhardt).
6. Kjeldatherm digestion tubes, 250 mL (Gerhardt).
7. Metal tube rack (Gerhardt).
8. Filter paper 5 × 8 cm approximately.
9. 1000 mL Glass Beaker.
10. 1000 mL volumetric flask, Class A.
11. Liquid dispenser with a resolution of 0.5 mL and capable of dispensing in a range of 2.5–25 mL, mod. Genius 1,605,006 (Walu).
12. 500 mL washing bottle.
13. Distilled water.
14. Sulfuric acid 96% for analysis, ISO (*see Note 2*).
15. Universal indicator of pH, solution for volumetric analysis.
16. Kjeldahl catalyst tablets: Kjeltabs 5 g K_2SO_4 + 0.5 g $CuSO_4 \times 5H_2O$ (Gerhardt).

17. Boric acid 2%. Dissolve 20 g of boric acid in 1 L of distilled water.
18. Sodium hydroxide solution 32% for Kjeldahl.
19. Sodium hydroxide pellets, ACS, reagent grade.
20. Sodium hydroxide 15%. Dissolve 180 g of sodium hydroxide pellets in 1.2 L of distilled water. Add several drops of universal pH indicator. It will be valid until the indicator turns from blue to yellow (*see Note 3*).
21. Hydrochloric acid 0.1 N PA, quality titrated solution.
22. Buffer solution pH 7.00 ± 0.02 .
23. Buffer solution pH 4.00 ± 0.02 .

2.5 Ash Determination

1. Muffle furnace, mod. RWF12–13 (Carbolite).
2. Analytical balance, with a resolution of 0.0001 g, mod. ME 614S (Sartorius).
3. Desiccator equipped with an effective dehydrator (Silica Gel with humidity indicator).
4. Socorex ACURA 825 Micropipette 100–1000 μL .
5. Porcelain crucibles of 25 mL, glazed inside and out, except base. Certificate according to DIN 12904. Withstands maximum temperature of 1000 °C.
6. Suitable forceps for handling porcelain crucibles.
7. Heat resistant gloves (*see Note 4*).
8. Drying oven with capacity to dry the samples at 105 ± 5 °C, mod. UFP 600 (Mettler).

3 Methods

Carry out all procedures at room temperature unless otherwise specified.

3.1 Sample Preparation for Analysis

The first step is to chop the sample into small cubes and homogenize it correctly [8]. Both chopping and homogenization are carried out with a professional mill KN 295 Knifetec (Foss) or a Homogeniser HM 294 (Foss) depending on the type/size of the sample. This step is very important in order to achieve good and representative results.

3.2 Moisture Determination

Moisture content is determined by oven drying (Mettler UFP 600, Schwabach, Germany) at 105 °C until constant weight, and is calculated as sample weight loss, according to ISO 1442 (1997) [9]. Figure 1 shows in a simplified and schematic way the steps of the moisture determination.

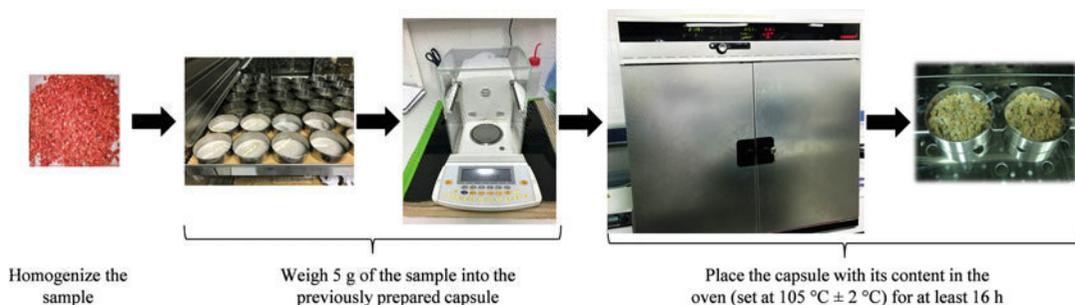


Fig. 1 Schematic and simplified representation of the steps of the moisture determination

1. Firstly, prepare the stainless steel capsules with sea sand and a glass rod (*see Note 5*). Then, the set of capsules, sea sand, and glass rod must be dried for at least 1 h in the oven (set at 105 °C ± 2 °C).
2. Before weighing the capsules, sea sand and glass rod must be at room temperature. To do this, they need to be placed in a desiccator (containing an efficient desiccant, such as silica gel) for 1 h.
3. Next, weigh the set (capsule, sea sand, and glass rod) on an analytical balance, with an accuracy of 0.0001 g.
4. Weigh 5 g of minced and homogenized sample into the capsule which has sea sand and a glass rod.
5. Mix the contents of the capsule (sample + sea sand) with the help of the glass rod (*see Note 6*).
6. Place the capsule with its content (sea sand + sample + ethanol) in the oven (set at 105 °C ± 2 °C) for at least 16 h (until reaching constant weight).
7. After the necessary time, remove the capsules with its contents from the oven and place them in the desiccator for 1 h (until reaching room temperature).
8. Finally, weigh the capsules and its content on an analytical balance, with an accuracy of 0.0001 g.
9. Expression of results
 - (a) Calculate the moisture content, as a percentage by mass, using the following equation:

$$\% \text{Moisture} = \left[\frac{(W_1 - W_2)}{(W_1 - W_0)} \right] \times 100$$

where:

- W_0 is the capsule, glass rod, and sea sand weigh (g).
- W_1 is the capsule containing the sample, glass rod, and sea sand weigh before drying (g).
- W_2 is the capsule containing the sample, glass rod, and sea sand weigh after drying (g).