Methods and Protocols in Food Science

Springer Protocols

Silvani Verruck
Eliane Teixeira Marsico *Editors*

Functional Meat Products

Humana Press

METHODS AND PROTOCOLS IN FOOD SCIENCE

Series Editor
Anderson S. Sant'Ana
University of Campinas
Campinas, Brazil

For further volumes:
http://www.springer.com/series/16556

Methods and Protocols in Food Science series is devoted to the publication of research protocols and methodologies in all fields of food science.

Volumes and chapters will be organized by field and presented in such way that the readers 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 will be clarified.

Functional Meat Products

Edited by

Silvani Verruck

Department of Food Science & Technology, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

Eliane Teixeira Marsico

Fluminense Federal University, Niterói, Brazil

Humana Press

Editors
Silvani Verruck
Department of Food Science & Technology
Federal University of Santa Catarina
Florianópolis, Santa Catarina, Brazil

Eliane Teixeira Marsico
Fluminense Federal University
Niterói, Brazil

ISSN 2662-950X ISSN 2662-9518 (electronic)
Methods and Protocols in Food Science
ISBN 978-1-0716-3572-8 ISBN 978-1-0716-3573-5 (eBook)
https://doi.org/10.1007/978-1-0716-3573-5

This Humana imprint is published by the registered company Springer Science+Business Media, LLC, part of Springer Nature.
The registered company address is: 1 New York Plaza, New York, NY 10004, U.S.A.

Paper in this product is recyclable.

Preface to the Series

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 basis are provided for each protocol. Aspects related to improvements in the protocols, adaptations, and further developments in the protocols may also be approached.

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 readers 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 a comprehensive and step-by-step procedures on how to perform that experiment. The next section brings the do's 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 complain 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, Brazil *Anderson S. Sant'Ana*

Preface

Meat production has tripled over the last four decades and increased 10% in the last 10 years. In 2020, meat production was around 328 million tons (Mt). Over the next decade, the worldwide consumption of meat proteins is projected to increase by 14, primarily driven by income and population growth. Thus, the global meat supply will expand over the projection period, reaching 374 Mt by 2030. Protein availability from beef, pork, poultry, and sheep meat is projected to grow by 5.9%, 13.1%, 17.8%, and 15.7%, respectively, by 2030. However, meat and by-product consumption are often related to non-transmissible chronic diseases, such as cardiovascular issues, diabetes, and intestinal and colorectal cancer. This relation occurs mainly because some kinds of meat and processed meat present a large amount of saturated fatty acids, cholesterol, sodium chloride, and other additives, such as nitrite and nitrates, that could be responsible for nitrosamine formation. Thus, the meat industry has been reviewing animal genetics and diets and also reformulating meat products in order to develop healthier formulations. The processed meat reformulation moves toward the decrease of fat, sodium, or cholesterol content. In addition, a better composition of unsaturated fatty acids, natural additives utilization, and even the incorporation of functional ingredients has been tested and stimulated.

Some fibers or prebiotics have been used to develop meat products with reduced saturated fat content, as they contribute to the stabilization of meat emulsions and improve the product's yield and texture. Prebiotics could also be selectively used as substrate in fermented meat products and, thus, could be considered an attractive strategy to increase healthiness by stimulations of beneficial bacteria, such as probiotics. Probiotics are able to produce health-improving compounds, usually via the hydrolysis of polysaccharides, proteins, and fats, creating biologically active compounds such as bioactive peptides, organic acids, vitamins, and conjugated linoleic acid. Additionally, enrichment of meat products with vitamins, unsaturated fatty acids, natural additives, and minerals are important approaches within the context of the development of functional meat products. These functional ingredients could have beneficial effects on human health while meeting consumer expectations for nutritionally improved meat products. On the other hand, there are several details to be observed in the reformulation of meat products with functional ingredients, including chemical, physical, microbiological, and sensory analyses stability.

The purpose of this book is to give a comprehensive introduction to methods and procedures related to the manufacture of functional meat products. To reach this goal, scientists from different disciplines like Food Engineering, Food Technology, Food Microbiology, Chemistry, Sensory Analyses, Pharmaceutics, and Nutrition will work in chapters to provide comprehensive protocols in this field. This book follows the highly successful Methods and Protocols in Food Science (MeFS) series format. All chapters include introductions to the respective topic, lists of all necessary materials and reagents, step-by-step, readily reproducible protocols, and notes giving tips on troubleshooting and avoiding pitfalls in the methodologies.

Chapter 1—Probiotic Fermented Meat Products—explores the world of probiotics in meat production. Readers will learn about the incorporation of beneficial bacteria into fermented meat products, promoting not only enhanced flavor but also potential health benefits. Chapter 2—Probiotic Emulsified Meat Products—delves into the development of emulsified meat products that incorporate these beneficial microorganisms. Readers will discover how to develop products that marry the qualities of emulsified meats with the health advantages of probiotics. Chapter 3—Prebiotic Meat Products—explores how prebiotic ingredients can be used in meat products to stimulate the growth of beneficial gut bacteria, resulting in improved health outcomes. Chapter 4—Symbiotic Fermented Meat Products—focuses on the synergy of probiotics and prebiotics. Readers will learn how to create fermented meat products that harness the power of both probiotics and prebiotics to enhance flavor and health benefits.

Chapter 5—Fermented and Structured Meat Products with Fibers for Reducing Fat Content—delves into the fascinating world of using fibers in meat products. Readers will discover how to create structured meat products that incorporate fibers, reducing fat content while maintaining texture and taste. Chapter 6—Emulsified Meat Product with Fibers for Reducing Fat Content—narrows the focus to emulsified meat products. Readers will explore how fibers can be used in emulsified meats to reduce fat content while retaining desirable qualities. In Chap. 7—Emulsified Meat Product with Oleogels for Reducing Fat Content—readers will learn how to incorporate oleogels into emulsified meat products to reduce fat content without compromising texture or taste.

Chapter 8—Analysis of Thiamine, Riboflavin, and Nicotinic Acid in Meat—describes insights into techniques for accurately measuring essential B-vitamins in meat products. In Chap. 9—Natural Additives in Meat Products as Antioxidants and Antimicrobials—readers will explore the use of natural additives in meat products, particularly for their roles as antioxidants and antimicrobials, helping to extend shelf life and ensure safety. Chapter 10—In Vitro and In-Model Evaluation of the Antimicrobial Activity of Lactic Acid Bacteria Protective Cultures to Replace Nitrite in Dry Fermented Sausages—discusses the intriguing possibility of replacing nitrite with protective cultures. Readers will learn about in vitro and in-model evaluation techniques for assessing the antimicrobial effects of these cultures in dry fermented sausages.

Chapter 11—Sodium Reduced Meat Products—is considered a critical health concern. Readers will discover methods and strategies for developing meat products with reduced sodium while preserving taste and safety. Chapter 12—Direct Method for Simultaneous Analysis of Cholesterol and Cholesterol Oxides by HPLC in Meat and Meat Products—provides a precise analytical approach to simultaneously measure cholesterol and cholesterol oxides in meat, aiding in nutritional assessment and quality control. Chapter 13—The Long-lasting Potential of the DNPH Spectrophotometric Method for Protein-derived Carbonyl Analysis in Meat and Meat Products—delves into a long-lasting method for analyzing protein-derived carbonyls in meat products, a valuable tool for quality control and research.

Finally, Chap. 14—Functional Molecules Obtained by Membrane Technology—explores membrane technology to obtain functional molecules from meat. Readers will discover how this innovative approach can yield valuable compounds for various applications. And Chap. 15—Bioactive Peptides Obtained from Meat Products—explores the world of bioactive peptides derived from meat products. Readers will learn about methods to isolate and utilize these peptides, potentially unlocking their health benefits.

The focus of this special volume is to address the latest relevant state-of-the-art protocols to manufacture functional meat products. In addition, this book combines as comprehensibly as possible well-established protocols and procedures being used by several laboratories in academia and industry. It will introduce the broad field of protocols that can be used for functional meat products production to Graduate Students, Postdoctoral Associates, and all researchers who are either still at the beginning of their academic careers or scientists who are in search of new challenges in a new field hitherto unfamiliar to them. In summary, this book covers a wide spectrum of topics within the realm of functional meat products, ranging from the incorporation of probiotics and prebiotics to analytical methods, innovative fat reduction techniques, and the utilization of natural additives and bioactive compounds. It provides a comprehensive guide for researchers and professionals in the food industry looking to explore and contribute to the development of healthier and more innovative meat products.

Florianópolis, Santa Catarina, Brazil ***Silvani Verruck***
Niterói, Brazil ***Eliane Teixeira Marsico***

Contents

Contributors

RUBEN AGREGÁN • *Centro Tecnológico de la Carne de Galicia, Parque Tecnológico de Galicia, Ourense, Spain*

GIORDANA DEMAMAN AREND • *Laboratory of Membrane Processes (LABSEM), Federal University of Santa Catarina, Florianopolis, RS, Brazil*

BAZHENA ARTEMOVA • *International Research Centre "Biotechnologies of the Third Millennium", Faculty of Biotechnologies (BioTech), ITMO University, Saint-Petersburg, Russia*

DENIS BARANENKO • *International Research Centre "Biotechnologies of the Third Millennium", Faculty of Biotechnologies (BioTech), ITMO University, Saint-Petersburg, Russia*

MIRELLA ROMANELLI VICENTE BERTOLO • *University of São Paulo (USP), São Carlos Institute of Chemistry (IQSC), São Carlos, SP, Brazil*

VANESSA BIASI • *Instituto Federal Catarinense – Campus Concórdia, Concórdia, SC, Brazil*

STANISLAU BOGUSZ JUNIOR • *University of São Paulo (USP), São Carlos Institute of Chemistry (IQSC), São Carlos, SP, Brazil*

YULIA BROYKO • *Department of Technology of Microbiological Synthesis, Saint-Petersburg State Institute of Technology, Saint-Petersburg, Russia*

JIAMING CAI • *Key Laboratory of Meat Processing and Quality Control, MOE, Jiangsu Synergetic Innovation Center of Meat Processing and Quality Control, Nanjing Agricultural University, Nanjing, PR China*

ANA KAROLINE FERREIRA IGNÁCIO CÂMARA • *Department of Food Engineering, Federal University of São João Del Rei, São João Del Rei, Minas Gerais, Brazil*

PAULO C. B. CAMPAGNOL • *Departmento de Tecnologia e Ciência de Alimentos, Universidade Federal de Santa Maria, Santa Maria, Brazil*

DANIEL RODRIGUES CARDOSO • *University of São Paulo (USP), São Carlos Institute of Chemistry (IQSC), São Carlos, SP, Brazil*

SUSANA CASAL • *LAQV-REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal*

KEILA NASCIMENTO CAVALCANTE • *University of São Paulo (USP), São Carlos Institute of Chemistry (IQSC), São Carlos, SP, Brazil*

CARLOS ADAM CONTE-JUNIOR • *Graduate Program in Food Science (PPGCAL), Institute of Chemistry (IQ), Federal University of Rio de Janeiro (UFRJ), Cidade Universitária, Rio de Janeiro, RJ, Brazil; Center for Food Analysis (NAL), Technological Development Support Laboratory (LADETEC), Federal University of Rio de Janeiro (UFRJ), Cidade Universitária, Rio de Janeiro, RJ, Brazil; Laboratory of Advanced Analysis in Biochemistry and Molecular Biology (LAABBM), Department of Biochemistry, Federal University of Rio de Janeiro (UFRJ), Cidade Universitária, Rio de Janeiro, RJ, Brazil; Faculty of Veterinary Medicine, Department of Food Technology, Fluminense Federal University (UFF), Niterói, RJ, Brazil; Graduate Program in Chemistry (PGQu), Institute of Chemistry (IQ), Federal University of Rio de Janeiro (UFRJ), Cidade Universitária, Rio de Janeiro, RJ, Brazil*

Rebeca Cruz • *LAQV-REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal*
Thais Lomonaco Teodoro da Silva • *School of Food Engineering, State University of Campinas, Campinas, São Paulo, Brazil*
Elizabeth Aparecida Ferraz da Silva Torres • *Department of Nutrition, School of Public Health, University of São Paulo (USP), São Paulo, SP, Brazil*
Vanessa Sales de Oliveira • *Department of Food Technology, Institute of Technology, University Federal Rural of Rio de Janeiro, Seropédica, RJ, Brazil*
Josemar Gonçalves de Oliveira Filho • *Brazilian Agricultural Research Corporation, Embrapa Instrumentation, São Carlos, SP, Brazil*
Camila de Souza Paglarini • *School of Architecture and Engineering, Mato Grosso State University and School of Nutrition, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil*
Ceres Mattos Della Lucia • *Graduate Program in Nutrition Science (PPGCN), Department of Nutrition and Health (DNS), Federal University of Viçosa (UFV), Laboratory of Vitamin Analysis (LAV), Viçosa, MG, Brazil*
Carolina E. Demaman Oro • *Department of Food Engineering, URI Erechim, Erechim, RS, Brazil*
Rubén Domínguez • *Centro Tecnológico de la Carne de Galicia, Avd. Galicia No. 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, Spain*
Maicon S. N. dos Santos • *Laboratory of Agroindustrial Processes Engineering (LAPE), Federal University of Santa Maria, Cachoeira do Sul, RS, Brazil*
Noemí Echegaray • *Centro Tecnológico de la Carne de Galicia, Ourense, Spain*
Vivian Feddern • *Embrapa Suínos e Aves, Concórdia, SC, Brazil*
Juana Fernandez • *IPOA Research Group (UMH-1 Grupo REVIV-Generalitat Valenciana), Dept Tecnología Agroalimentaria, Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández, Orihuela, Spain*
Manuela Fernández • *Department of Food Technology, Veterinary Faculty, Complutense University of Madrid (UCM), Madrid, Spain*
Laureano Frizzo • *Laboratory of Food Analysis Med. Vet R. Dalla Santina, Institute of Veterinary Science (ICiVet Litoral), National University of the Litoral (UNL)- National Council of Scientific and Technical Research (UNL/CONICET), Esperanza, Province of Santa Fe, Argentina; Faculty of Veterinary Science, Department of Public Health, National University of the Litoral, (UNL), Esperanza, Province of Santa Fe, Argentina*
Eva Hierro • *Department of Food Technology, Veterinary Faculty, Complutense University of Madrid (UCM), Madrid, Spain*
Xavier F. Hospital • *Department of Food Technology, Veterinary Faculty, Complutense University of Madrid (UCM), Madrid, Spain*
Eduardo Huber • *Instituto Federal Catarinense – Campus Concórdia, Concórdia, SC, Brazil*
Ming Ju • *Key Laboratory of Meat Processing and Quality Control, MOE, Jiangsu Synergetic Innovation Center of Meat Processing and Quality Control, Nanjing Agricultural University, Nanjing, PR China*
Vicky Lilge Kawski • *Embrapa Suínos e Aves, Concórdia, SC, Brazil*
Malikakhon Kurbonova • *International Research Centre "Biotechnologies of the Third Millennium", Faculty of Biotechnologies (BioTech), ITMO University, Saint-Petersburg, Russia*

Elena Lemeshonok • *International Research Centre "Biotechnologies of the Third Millennium", Faculty of Biotechnologies (BioTech), ITMO University, Saint-Petersburg, Russia*

José Manuel Lorenzo • *Centro Tecnológico de la Carne de Galicia, Avd. Galicia No. 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, Spain; Facultade de Ciencias, Área de Tecnoloxía dos Alimentos, Universidade de Vigo, Ourense, Spain*

José Manuel Lorenzo • *Centro Tecnológico de la Carne de Galicia, Avd. Galicia No. 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, Spain; Facultade de Ciencias, Área de Tecnoloxía dos Alimentos, Universidade de Vigo, Ourense, Spain*

Claudio Malaghini • *Department of Food Science and Technology, Federal University of Santa Catarina, Florianopolis, SC, Brazil*

Cynthia Farias Manassi • *Department of Food Science and Technology, Universidade Federal Santa Catarina (UFSC), Florianópolis, SC, Brazil*

Sergio B. Mano • *Department of Food Technology, Fluminense Federal University (UFF), Nitéroi, RJ, Brazil*

Eliane Teixeira Mársico • *Faculty of Veterinary Medicine, Department of Food Technology, Fluminense Federal University (UFF), Niterói, RJ, Brazil*

Maria Lúcia Guerra Monteiro • *Graduate Program in Food Science (PPGCAL), Institute of Chemistry (IQ), Federal University of Rio de Janeiro (UFRJ), Cidade Universitária, Rio de Janeiro, RJ, Brazil; Center for Food Analysis (NAL), Technological Development Support Laboratory (LADETEC), Federal University of Rio de Janeiro (UFRJ), Cidade Universitária, Rio de Janeiro, RJ, Brazil; Laboratory of Advanced Analysis in Biochemistry and Molecular Biology (LAABBM), Department of Biochemistry, Federal University of Rio de Janeiro (UFRJ), Cidade Universitária, Rio de Janeiro, RJ, Brazil*

Sinara Teixeira do Brasil Morais • *University of São Paulo (USP), São Carlos Institute of Chemistry (IQSC), São Carlos, SP, Brazil*

Paulo E. S. Munekata • *Centro Tecnológico de la Carne de Galicia, Parque Tecnológico de Galicia, San Cibrao das Viñas, Spain*

Lívya Alves Oliveira • *Graduate Program in Nutrition Science (PPGCN), Department of Nutrition and Health (DNS), Federal University of Viçosa (UFV), Laboratory of Vitamin Analysis (LAV), Viçosa, MG, Brazil*

Mirian Pateiro • *Centro Tecnológico de la Carne de Galicia, Avd. Galicia No. 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, Spain*

José Ángel Pérez-Álvarez • *Department Tecnología Agroalimentaria, IPOA Research Group (UMH-1 Grupo REVIV-Generalitat Valenciana), Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández, Orihuela, Spain*

Marise Aparecida Rodrigues Pollonio • *School of Food Engineering, State University of Campinas, Campinas, São Paulo, Brazil*

Katia Rezzadori • *Department of Food Science and Technology, Federal University of Santa Catarina, Florianopolis, SC, Brazil*

Marcelo Rosmini • *Laboratory of Food Analysis Med. Vet R. Dalla Santina, Institute of Veterinary Science (ICiVet Litoral), National University of the Litoral (UNL)- National Council of Scientific and Technical Research (UNL/CONICET), Esperanza, Province of Santa Fe, Argentina; Faculty of Veterinary Science, Department of Public Health, National University of the Litoral, (UNL), Esperanza, Province of Santa Fe, Argentina*

Tatiana Saldanha • *Department of Food Technology, Institute of Technology, University Federal Rural of Rio de Janeiro, Seropédica, RJ, Brazil*

Geni Rodrigues Sampaio • *Department of Nutrition, School of Public Health, University of São Paulo (USP), São Paulo, SP, Brazil*
Eva Maria Santos • *Área Académica de Química, Universidad Autónoma del Estado de Hidalgo, Mineral de la Reforma, Mexico*
Irina Shestopalova • *Department of Technology of Microbiological Synthesis, Saint-Petersburg State Institute of Technology, Saint-Petersburg, Russia*
M. Madalena C. Sobral • *LAQV-REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Ciências Químicas, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal*
Alfredo Teixeira • *Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal; Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Bragança, Portugal*
Marcus V. Tres • *Laboratory of Agroindustrial Processes Engineering (LAPE), Federal University of Santa Maria, Cachoeira do Sul, RS, Brazil*
Nikita Tyutkov • *International Research Centre "Biotechnologies of the Third Millennium", Faculty of Biotechnologies (BioTech), ITMO University, Saint-Petersburg, Russia*
Silvani Verruck • *Department of Food Science and Technology, Universidade Federal Santa Catarina (UFSC), Florianópolis, SC, Brazil*
Lujuan Xing • *Key Laboratory of Meat Processing and Quality Control, MOE, Jiangsu Synergetic Innovation Center of Meat Processing and Quality Control, Nanjing Agricultural University, Nanjing, PR China*
Jian Zhang • *Key Laboratory of Meat Processing and Quality Control, MOE, Jiangsu Synergetic Innovation Center of Meat Processing and Quality Control, Nanjing Agricultural University, Nanjing, PR China*
Wangang Zhang • *Key Laboratory of Meat Processing and Quality Control, MOE, Jiangsu Synergetic Innovation Center of Meat Processing and Quality Control, Nanjing Agricultural University, Nanjing, PR China*

Chapter 1

Probiotic Fermented Meat Products

Ruben Agregán, Marcelo Rosmini, José Ángel Pérez-Álvarez, Paulo E. S. Munekata, Laureano Frizzo, Paulo C. B. Campagnol, and José M. Lorenzo

Abstract

The fermentation of meat is an ancient culinary tradition worldwide used mainly with the intention of extending meat shelf life and diversifying. Plenty of products with their respective recipes have been developed throughout the history of civilization. Spain is a country with historical tradition in the production of fermented meat products, highlighting chorizo and salchichón. Specifically, the latter can be divided into different varieties according to aspects, such as size. Thus, products, such as longaniza, fuet, secallona, or didalets, can be classified and named according to the length and width of the piece. The ingredients used for elaboration are practically the same between these salchichónes. In the present chapter, the production of fuet is described in depth since it represents one of the most traditional and consumed fermented meat products in Spain and it is also being internationalized to other countries. On the other hand, the addition of probiotic cultures to meat dough is increasingly practiced, which has potential health benefits. Therefore, the production of fuet with probiotic microorganisms might help to develop novel and healthy alternatives to the traditional recipe. Ingredients including pork lean and belly, spices and other additives incorporated in the form of commercial mixes, and starter and probiotic cultures are used in the elaboration of the fuet proposed in this chapter, throughout different steps, which can be classified as mincing, mixing, stuffing, fermentation, curing, and conservation.

Key words Fermented meat product, Fermented sausage, Probiotic, Fuet, Elaboration process

1 Introduction

The elaboration of fermented meat products is a culinary tradition perpetuated over time by generations in different parts of the world, such as Europe, where a wide and varied offer of these products can be found [1]. Although the technology of these products has undergone significant modifications throughout the history of humankind [2], the purpose of fermenting meat has always been the same, extending shelf life and diversifying [3]. The development of fermented meat products involves dynamic and complex chemical processes, in which lactic acid

Silvani Verruck and Eliane Teixeira Marsico (eds.), *Functional Meat Products*,
Methods and Protocols in Food Science, https://doi.org/10.1007/978-1-0716-3573-5_1,

bacteria (LAB) stand as the main muscle-transforming microorganisms, causing the acidification of the medium. This drop in pH helps to stabilize the product, delaying deterioration processes and preventing the development of pathogenic bacteria. In addition, acidification positively impacts on sensory attributes, increasing the final product acceptance [1].

The fermentation of meat can be done in two ways, allowing the indigenous muscle microflora to act alone or using predefined microorganisms (known as starter cultures) to initiate and carry out the transformation processes. These starter cultures mainly consist of one or several LAB species, micrococci, and staphylococci [4], but also yeast and molds can be used [5]. They are specifically designed to meet the food safety criteria specified by the regulatory entity and the technological and organoleptic specifications of the company. In this way, the fermentation can be controlled, and the process standardized, yielding safe and high-quality meat products [6]. The current market trend towards healthier products has led to the research of other microbial cultures capable of exerting health benefits. In this context, special attention has been paid to probiotics, living organisms capable of modifying the gut microbiome and improving health when consumed in adequate amounts [7]. Immunomodulatory effects and anticancer, antimicrobial, antidiabetic, and anti-inflammatory properties have been associated with the consumption of these microorganisms [8–12].

Different studies have been searching for good probiotic candidates to be used in the preparation of fermented meat products (Fig. 1). Strains such as *Bifidobacterium longum* KACC 91563 [13], *Enterococcus faecium* CECT 410 [14], *Lacticaseibacillus casei* ATCC 393 [15], *Lactobacillus paracasei* DTA83 [16], *Lactobacillus rhamnosus* LOCK900 [17], *Lactobacillus acidophilus* CRL1014 [18], and *Lactobacillus sakei* 23 K [19] have been recently assessed in this regard, showing a good ability to produce quality fermented meat products since they are able to satisfactorily colonize the meat dough, reach a reasonably high number of counts, and barely affect sensory attributes, pH, and oxidative status [7]. As can be seen, there are many potential probiotic cultures to be used in the development of fermented meat products, which opens the door to multiple industrial and commercial possibilities [20].

For the elaboration of a fermented meat product, different and varied recipes can be followed since there are innumerable products of this type with very different characteristics, linked to geographical areas of the world [21, 22]. In Spain, there is a long tradition of making these food products and varieties, such as chorizo and salchichón, which can be tasted throughout the different territories of this country. Several classes of salchichón can be found according to parameters, such as size. Thus, products, such as longaniza, fuet, secallona, or didalets, with different lengths and widths are

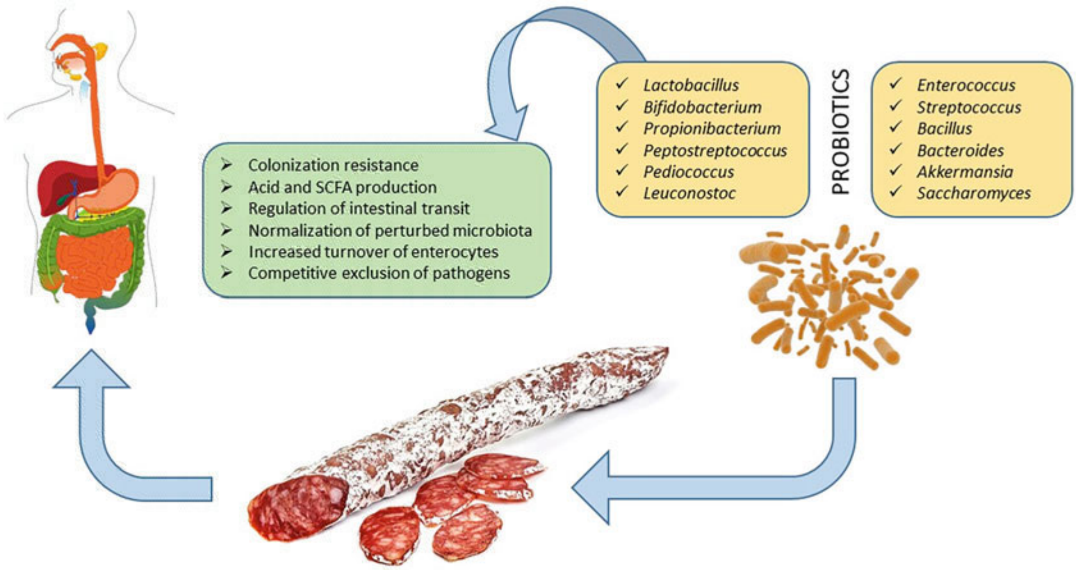

Fig. 1 Potential health benefits of probiotic fuet consumption and possible bacteria involved [24, 25]. *SCFA* short-chain fatty acid

commercialized. In this chapter, we selected the "fuet" as the base product to show its production process in depth. Fuet is a traditional product from the region of Catalonia widely consumed in Spain. Only in 2019 that consumers of this country spent more than 250 million dollars on this meat product and longaniza. In addition, fuet product is becoming international, and its consumption has spread to other neighboring countries such as France and also to the entire European continent and the United States, where it is prized for its presumed high-quality ingredients, exceptional flavor, and superior wholesomeness compared to similar Mediterranean-type sausages [23].

2 Materials

2.1 Ingredients

Fuet is made mainly with pork and fat. In addition, salt, spices, and sugar are also used. Different preparations of this fermented sausage are possible, so in order to avoid conflicts between the multiple existing formulations, we have decided to compile some of the most relevant recipes recently published in the scientific literature to develop our own concept of fuet. Pork lean is a fundamental part of the traditional recipe, but other meats are currently replacing pork in some novel manufacturing protocols. After chopping and blending the lean and fat, salt and ground pepper are added. Other species such as garlic can also be incorporated. These seasonings work as flavor enhancers and can help in the stabilization process by

Table 1
Ingredients and proportions in the elaboration of the probiotic fuet

Ingredients	Proportion (%)
Pork lean	60
Pork fat	30
Water	4
Commercial mix	4
Commercial starter culture	1.98
Commercial probiotic strain	0.02

Commercial mix: salt, dextrin, dextrose, stabilizer (sodium phosphate (E-451)), spices and spice extract, flavor, antioxidants (sodium ascorbate (E-301) and sodium citrate (E-331)), and preservatives (potassium nitrate (E-252) and sodium nitrite (E-250)). Starter culture: *Pediococcus* (50%), *Staphylococcus xylosus* (25%), and *Staphylococcus carnosus* (25%). Probiotic strain: LGG® (*Lacticaseibacillus rhamnosus* GG). Data are based on the studies carried out by Bis-Souza et al. [29], Zamora et al. [30], and Peñaranda et al. [31]

exhibiting antioxidant and antimicrobial properties [26–28]. Differences between fuets, both commercial and homemade, can be found at this point of preparation. The type of meat and the fermentation process also have a significant influence on the final product.

Other ingredients, including stabilizer (e.g., phosphate), antioxidants (e.g., ascorbate and citrate), preservatives (e.g., nitrate and nitrite), dextrin, dextrose, and flavorings, are also added to the meat matrix. These compounds, together with the spices, are usually incorporated in the form of commercial mixes. A starter culture consisting different type of species, including LAB, and a probiotic culture are then inoculated. Finally, the formed dough needs to be completed with water up to a certain percentage of humidity. Artificial pig casings are used to stuff the dough obtained, but natural pork casings are also commonly used, and a food-grade *Penicillium candidum* mold is applied to the surface of sausage to protect it from the invasion of spoilage molds during storage. Moreover, it adds a touch of flavor and extra aroma to the final product. Finally, fermentation and drying processes complete the production protocol. Ingredients and proportions in the preparation of the probiotic fuet are detailed in Table 1.

There are many commercial mixes available on the market, but we suggest using the one sold by Catalina Food Solutions S.L. (El Palmar, Murcia, Spain) [30]. This mix is made up of salt, dextrin, dextrose, stabilizer (sodium phosphate (E-451)), spices and spice extract, flavor, antioxidants (sodium ascorbate (E-301) and sodium citrate (E-331)), and preservatives (potassium nitrate (E-252) and sodium nitrite (E-250)).

Regarding the starter culture, the commercial mix used by Zamora et al. [30] (Microsan-R), also from Catalina Food Solutions S.L. (El Palmar, Murcia, Spain), was chosen for the elaboration of the probiotic fuet. The genus *Pediococcus* at a concentration of 50% and the species *Staphylococcus xylosus* and *Staphylococcus carnosus* at concentrations of 25% each compose the starter culture. On the other hand, the probiotic strain LGG® (*Lacticaseibacillus rhamnosus* GG), marketed by the company Chr. Hansen (Hørsholm, Denmark) (*see* **Note 1**) and successfully tested in the preparation of a salchichón [29], was the one chosen to colonize the fuet. Finally, as previously mentioned, a layer of *Penicillium candidum* spores is applied to the product after stuffing. There are different commercial brands on the market that provide this mold. In this case, we propose the one marketed by the company Danisco S.A. (Barcelona, Spain), according to the elaboration carried out by Marcos et al. [32].

2.2 Equipment

The equipment for making fuet is an essential part of the production protocol since inadequate material can lead to undesirable results. Thus, we have suggested a series of elements and brands that can adequately satisfy the needs of manufacturers during the different stages of production (Table 2). Photos of this machinery are shown in Fig. 2.

A mincing machine is the first piece of equipment necessary for the fuet production process as it allows the chopping of meat and fat, which will form the base of the sausage. For this, a refrigerated mincer from La Minerva di Chiodini Mario (Bologna, Italy) with a 6 mm mincing plate can be used. For fine grinding and mixing of both raw materials, along with the commercial mixes of additives and microorganisms (starter culture and probiotic strain), an Industrial Fuerpla (Benetusser, Valencia, Spain) vacuum grinder is

Table 2
List of suitable industrial equipment for the elaboration of the probiotic fuet and the corresponding brands (*see* Notes 2 and 3)

Equipment	Brand
Mincing machine	La Minerva di Chiodini Mario (Bologna, Italy)
Vacuum grinder	Industrial Fuerpla (Benetusser, Valencia, Spain)
Semiautomatic stuffer	Sia Suministros Industriales (las Torres de Cotillas, Murcia, Spain)
Sausage tying machine	Andher-*Comercial Eliseo Andújar S.L.* (Alcázar de San Juan, Ciudad Real, Spain)
Air-drying chamber	–
Conservation chamber	–

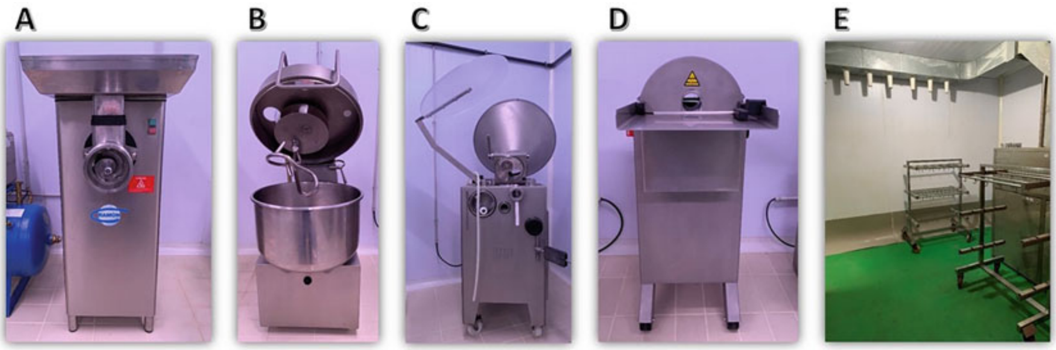

Fig. 2 Suggested equipment for the elaboration of a probiotic fuet. (**a**) mincing machine; (**b**) vacuum grinder; (**c**) semiautomatic stuffer; (**d**) sausage tying machine; (**e**) air-drying chamber

recommended. A conventional or industrial refrigerator will be necessary, depending on the amount of dough produced, to store it and allow the compaction to occur. Stuffing can be performed using a Sia Suministros Industriales (Las Torres de Cotillas, Murcia, Spain) semiautomatic stuffer and a 45 mm diameter artificial casings (Edicas, Salamanca, Spain). The sausages formed can be divided and tied using an Andher sausage tying machine (Alcázar de San Juan, Ciudad Real, Spain). Finally, the fermentation and drying processes can be carried out in an air-drying chamber. After elaboration, the product can be moved to another automated chamber to preserve it until consumption.

3 Methods (*See* Note 4)

Fuet requires a strict production protocol and good raw materials (lean meat and belly) to obtain a quality product. Once these elements are minced and mixed with the commercial mix and both the starter and probiotic cultures, the dough formed is stuffed, fermented and dried, and conserved (Fig. 3). This highly summarized production procedure consists of a series of detailed stages that will be described in depth in the following paragraphs.

3.1 Chopping of Raw Materials

The first step when making fuet is to obtain good-quality raw materials. A product made with meat and fat of little aptitude for the preparation of sausages will affect the quality of the product. The classical recipe for fuet uses pork lean and pork belly. However, recently modifications of this model recipe have been appearing, which attempt to diversify this product by incorporating proteins from different animals. Meat from animals, such as goat, sheep, beef, and other less common, such as duck, foal, or game, can be used in the production of salchichón [33–35]. In our case, both the meat and fat for making fuet will be from pork.

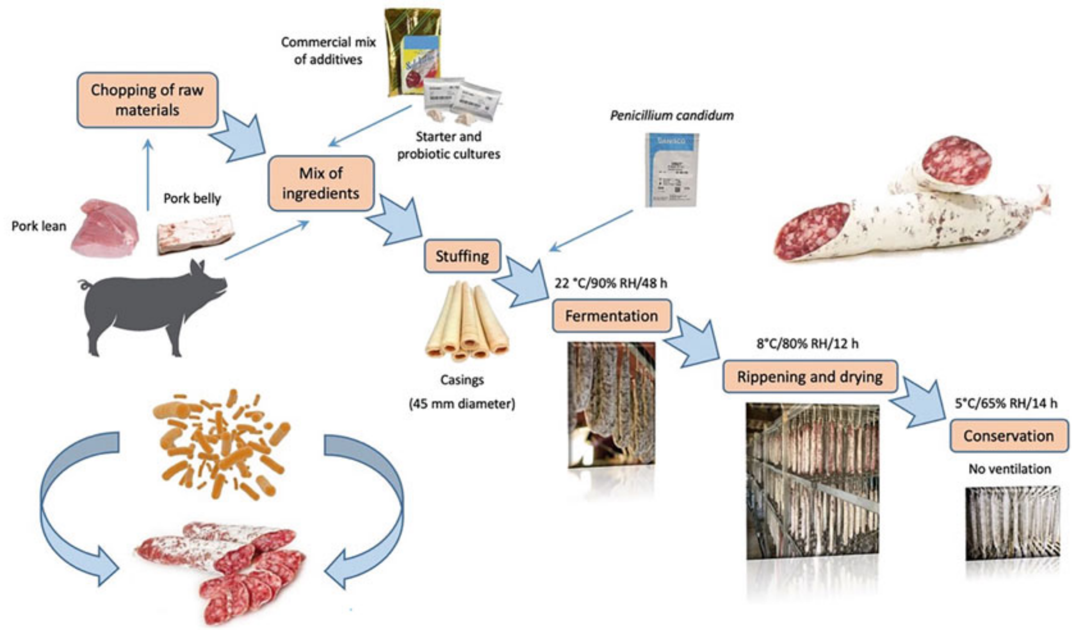

Fig. 3 Production scheme of probiotic fuet

The previously refrigerated pork lean and pork belly are cut into chunks of between approximately 10 × 10 and 20 × 20 cm to allow their easy passage through the mincer tube. The meat and fat are placed separately in the mincer's loading hopper and are pushed by an auger towards the hole that ends in a perforated disc that grinds the raw material. The chopped lean and fat are then collected in a clean stainless steel container. In this operation, the sharpness of the blades must be adequate; otherwise, the mincing may be poor, causing tears in the meat and overheating. This leads to problems in the ripening and drying stage, giving rise to fuets with poorly defined short surfaces.

3.2 Mix of Raw Materials with Additives and Starter and Probiotic Cultures

After the mincing process, the lean and fat are properly mixed under refrigerated (< 4 °C) vacuum. The absence of air is essential to prevent subsequent problems, such as discoloration and a higher development of microorganisms. At this point in processing, the commercial mix of additives in powdered form, incorporating salt, spices and spice extract, dextrin, dextrose, and flavoring, along with a stabilizer, antioxidants, and preservatives, is poured over the meat mixture and fat. This operation should last the time enough to allow the formation of the most uniform paste as possible. Around 5 min would be needed to process 20 kg of dough. During the mixing process, both the starter and probiotic cultures are added. The moment and the order of addition are indifferent. Specifically, the commercial starter culture will be incorporated in the form of lyophilized powder (commercial presentation), being previously