Chapter 2. Sourdough microbiota diversity in Southern Europe

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Abstract

Sourdough is a complex microbial ecosystem mainly characterized by the leavening action of acid-tolerant yeasts and lactic acid bacteria (LAB). Sourdough and its derived bakery goods are worldwide staple foods, particularly in the Mediterranean diet. The microbial diversity of traditional sourdoughs has been related, at different extents, to the raw matrices, the working environment, as well the conditions faced during food processing. Though microbial region specificity is claimed to improve the unique qualities of traditional products, a clear relationship between typical sourdough and its associated microbiota is still an open question. In this chapter, we provide an overview of the state-of-art of sourdough microbial diversity with a focus on Italian and Portuguese food productions. In particular, we discuss the main traditional National sourdough bread(s) and other sourdough-based baking products, and analyzing the microbial composition and pointing out technological as well as economic, social and cultural issues. By examining the traditional features of sourdoughs from these countries, we can gain valuable insights into the microbial ecology and evolution of sourdough fermentation and uncover potential connections between microbiota composition and sourdough quality.

Keywords
Sourdough, Breadmaking, Italy, Portugal, Traditional bread, Traditional bakery goods

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1. Introduction

Sourdough is a mixture of flour (or flours) and water fermented by endogenous complex microbial communities, mainly including yeasts and lactic acid bacteria (LAB), with a prevalence of heterofermentative lactobacilli (De Vuyst et al., 2016). These microorganisms are pivotal on shaping sourdough's unique sensory attributes, texture, nutritional features and derivative products (De Vuyst et al., 2023; Gänzle, 2014; Ma et al., 2021). Noteworthy, it is also the microbial contribution that enhance the safety and shelf-life of sourdough-based foods by synthesizing several antimicrobial compounds useful to contrast the development of spoilage filamentous fungi and/or pathogens (Axel et al., 2016; Quattrini et al., 2019; Russo et al., 2017). More recently, the scientific interest has also moved towards the improvement of the functional properties suggesting the design of selected LAB and yeasts targeting the optimization of sourdough bioprocesses (Gobbetti et al., 2019; Graça et al., 2021; Ribet et al., 2023). Accordingly, the occurrence of postbiotic-like components synthesized through sourdough fermentation and/or released during the baking process is opening new perspectives in the effort of linking microbial resources to specific functions, with a particular interest in metabolites providing human health benefits (Akamine et al., 2023; Lau et al., 2021; Pérez-Alvarado et al., 2022).

Traditional sourdoughs are obtained by spontaneous fermentation and further subjected to back slopping (type-I sourdough), thus offering a rich and dynamic ecosystem in terms of microbial diversity that is related, at different extent, to the raw matrices, the working environment, as well the conditions faced during food-processing (Calabrese et al., 2022; Novotni et al., 2021). In general, the most prevalent LAB species reported in sourdough are *Fructilactobacillus sanfranciscensis*, *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Companilactobacillus paralimentarius*, *Limosilactobacillus fermentum*, *Pediococcus pentosaceus*, *Leuconostoc* spp. and *Weissella* spp., while *Saccharomyces cerevisiae*, *Candida humilis*, *Kluyveromyces marxianus*, *Pichia* spp., *Wickerhamomyces anomalus* and *Torulaspora delbrueckii* the most commonly found yeast species (recently reviewed by De Vuyst et al., 2023). Microbial region specificity is claimed to improve the unique qualities of traditional products (Capozzi et al., 2012). However, a clear relationship between a typical sourdough and its associated microbiota is still an open question (De Vuyst et al., 2014). Elucidation can be deduced from a comprehensive systematic review analyzing 1230 studies based on thirty years of knowledge on sourdough fermentation (Arora et al., 2021). Moreover, a meta-analysis of 583 sourdough-related literature articles in the period 1999–2017 based on the microbial diversity and process condition revealed that sourdoughs were mainly characterized by the presence of *S. cerevisiae* or *C. humilis* and that the simultaneous presence of both yeasts seemed to be associated with the occurrence of the LAB *Fr. sanfranciscensis* (Van Kerrebroeck et al., 2017). Accordingly, sourdough often harbors only one or two yeast species. For instance, Italian bakery sourdoughs often displayed *C. humilis* and/or *S. cerevisiae* as dominating yeasts (De Vuyst et al., 2016). Recently, a comprehensive study has been performed to investigate how the microbial diversity of sourdough varies across and between Continents. Interestingly, this study suggested that geographical location minimally influences microbial communities, while the diversity likely depends on how the sourdough was obtained and maintained over time (Landis et al., 2021). In agreement, a recent study showed that the bacterial and fungal composition of wheat grain and flour was poorly represented in mature sourdough, indicating that selection by this ecological niche appears as the main driver of the mature sourdough microbial composition (von Gastrow et al., 2023).

Sourdough fermentation has played a pivotal role in the history and nutrition of humans. With its rich culinary history and diverse cultural heritage, Southern Europe offers a captivating landscape to explore the microbiota diversity of sourdoughs in this geographical region (Bonaccio et al., 2022).

This chapter aims to delve into the intricacies of sourdough microbiota in Southern Europe, unravelling the microbial players responsible for the distinct features of sourdough and bakery products, by providing an overview of the state-of-art in Italy and Portugal. By examining the traditional features of sourdoughs from these countries, we can gain valuable insights into the microbial ecology and evolution of sourdough fermentation, as well as uncover potential connections between microbiota composition and sourdough quality.

2. Traditional Italian and Portuguese sourdough breads and other sourdough-based baking products

The scientific, social and economic relevance of Southern Europe sourdough fermentation is widely testified by the importance of these products among the Protected Geographical Indication (PGI), a sign that allows associating unique quality features to a specific geographical origin in the framework of the intellectual property law (Capozzi et al., 2012). As listed in **Table 1**, three Italian breads have received the Protected Designation of Origin (PDO), namely *Pane di Altamura*, *Pagnotta del Dittaino*, and *Pane Toscano*, while a total of 18 Italian and Portuguese bakery products are recognized as Protected Geographical Indication (**Table 1**). Outside the European Community (EC) Regulation, Traditional Agri-Food Products (TAP) is an official approval for traditional Italian regional food products similar to the Protected Geographical Indication (PGI) status of the European Union (EU). TAPs encompass hundreds of bread, pastry, cakes, biscuits and other baker's wares diversified among all Italian Regions "*whose method of processing, preservation and maturation have been consolidated over time*" (https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/17979). The high number of sourdough-fermented products recorded in Italy is probably due to the existence of strong differences within the Italian territory, including geographical, historical and cultural concerns, which are reflected in a wide range of traditional products. Moreover, the artisanal features of this kind of food, the employment of traditional recipes, as well as the small-scale production are consistent with their local more than global availability.

Table 1. Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) in the field of bakery products in Italy and Portugal (Source: eAmbrosia)

Name	**Product category**	**File Number**	**Type**	**Country**	**Status**	**Date of Registration**
Coppia Ferrarese	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-IT-0120	PGI	Italy	Registered	18/10/2001
Pane di Altamura	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PDO-IT-0136	PDO	Italy	Registered	19/07/2003
Ricciarelli di Siena	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-IT-0666	PGI	Italy	Registered	19/03/2010
Pastel de Chaves	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-PT-1126	PGI	Portugal	Registered	27/05/2015
Amêndoa Coberta de Moncorvo	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-PT-02235	PGI	Portugal	Registered	16/03/2018
Focaccia di Recco col formaggio	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-IT-0944	PGI	Italy	Registered	14/01/2015
Torrone di Bagnara	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-IT-1101	PGI	Italy	Registered	14/08/2014
Piadina Romagnola / Piada Romagnola	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-IT-1067	PGI	Italy	Registered	04/11/2014
Folar de Valpaços	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-PT-01392	PGI	Portugal	Registered	14/02/2017
Pane di Matera	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-IT-0372	PGI	Italy	Registered	22/02/2008
Ovos Moles de Aveiro	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-PT-0518	PGI	Portugal	Registered	08/04/2009
Pagnotta del Dittaino	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PDO-IT-0577	PDO	Italy	Registered	18/06/2009
Liquirizia di Calabria	Class 1.8. Other products of Annex I of the Treaty (spices etc.), Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PDO-IT-0644	PDO	Italy	Registered	25/10/2011
Panforte di Siena	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-IT-0795	PGI	Italy	Registered	22/05/2013
Pastel de Tentúgal	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-PT-0938	PGI	Portugal	Registered	04/09/2013

Pastel de Tentúgal	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-PT-0938-AM01	PGI	Portugal	Applied	
Pane casareccio di Genzano	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-IT-1553	PGI	Italy	Registered	25/11/1997
Cantuccini Toscani / Cantucci Toscani	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-IT-01290	PGI	Italy	Registered	26/01/2016
Fogaça da Feira	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-PT-01342	PGI	Portugal	Registered	14/06/2016
Pane Toscano	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PDO-IT-01016	PDO	Italy	Registered	04/03/2016
Pão de Ló de Ovar	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-PT-01341	PGI	Portugal	Registered	24/08/2016
Pampapato di Ferrara / Pampepato di Ferrara	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-IT-01323	PGI	Italy	Registered	08/12/2015
Südtiroler Schüttelbrot / Schüttelbrot Alto Adige	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-IT-02392	PGI	Italy	Registered	24/07/2020
Sebadas / Seadas / Sabadas / Seattas / Savadas / Sevadas di Sardegna	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares, Class 2.5. Pasta	PGI-IT-02834	PGI	Italy	Published	---
Pampepato di Terni / Panpepato di Terni	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-IT-02467	PGI	Italy	Registered	23/10/2020
Caralhotas de Almeirim	Class 2.3. Bread, pastry, cakes, confectionery, biscuits and other baker's wares	PGI-PT-02622	PGI	Portugal	Applied	---

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For example, flat breads include a multitude of ancient and worldwide breads ranging from a few millimeters to a few centimeters in thickness. In a recent survey, a total of 143 different flat bread types were found to be produced in 9 countries of the Mediterranean area, of which 75 in Italy (Pasqualone et al., 2022). Apart from what above-reported, other reasons could explain the high occurrence of these products. Indeed, in Italy, they are considered as a delicacy, thus admitting many variations on a regional basis, including pain, garnished and fried products, while in other areas, they are staple foods consumed on a daily basis (Pasqualone et al., 2022). Among these, *Focaccia* is a common Italian flat bread, typically consumed as street food. Even if some differences among different traditional recipes can be reported, the production process generally includes kneading a blend of soft and durum wheat flour with water, fatty substances, yeast and salt, as well the addition of other ingredients, such as fresh cherry tomatoes, olives, dry oregano and some additional oil (Bavaro et al., 2021).

Italian traditional bakery goods also include some traditional sweet-leavened products mainly consumed in occasion of religious feasts (*i.e*. *Panettone* and *Pandoro* or *Colomba* for Christmas and Easter, respectively) and some snacks for breakfast like *Brioches* and *Cornetti* (Palomba et al., 2011). *Pandoro* originates from the city of Verona is typically frustum shaped with a star section, whose main ingredients are flour, sugar, eggs and butter (Lattanzi et al., 2013). *Colomba,* which takes its name from the typical shape of a dove, is decorated with glaze, almonds and granulated sugar (Raimondi et al., 2017). These products are characterized by the employment of sourdough, the so-called mother-sponge or mother-dough, kept active by continuous refreshments (back sloppings). Fermentation times are usually longer and performed at lower temperatures than what is used for other bakery products (Montanari et al., 2014). However, even if considered traditional Italian goods, they are produced at both industrial and artisanal levels and have a national and international diffusion (Vernocchi et al., 2004).

Although wheat is the most commonly used cereal in breadmaking in Europe, other cereals and pseudocereals (such as amaranth, quinoa and buckwheat) are used, and new formulations and baking products are continuously being developed worldwide to meet the needs of contemporary consumers (Novotni *et al*., 2023). Portugal has a wide multiplicity of traditional breads and *broa* or *broa de milho* – a traditional Portuguese bread made of regional maize made and rye flours – is widely consumed in many regions of Portugal, especially in the Northern.

An inventory (DGADR, 2001) of the types of the traditional *broas* and other Portuguese breads and baking specialties (in addition to other traditional Portuguese agricultural products) was created by DGADR (2023), in partnership with FPCG (2023) and MINHA TERRA (2023). These traditional Portuguese specialties are summarized in **Table 2**, even though many other traditional bakery goods may be found around the country.

Table 2. Traditional breads and other baking goods from different geographical regions in Portugal (DGADR, 2001)

Name (and other designations)	**Flour or flours**	**General characteristics**	**Main geographical region or municipality**
Bola com Torresmos (*Bolo de Torresmos*; *Torta de Torresmos*)	Wheat	More or less spherical shaped balls, about the size of a clenched fist. Wheat, salt, mother-dough (*massa-mãe*) and water, sometimes made with the addition of lard to the dough and stuffed with greaves. Variants: with dried fermented sausages (*linguiça*) instead of greaves.	Alentejo region
Bola de Carne à Lavrador and *Bola de Bacalhau*	Wheat	Rectangle shape, brownish color. Wheat, salt, mother-dough (*massa velha*) and water, stuffed with smoked meat or salted codfish (*bacalhau*) and soft texture.	Municipality of Marco de Canaveses. North region
Bola de Centeio de Barroso (*Bola Centeia*)	Rye	Flat base and round or oval shape. Rye, salt, mother-dough (*massa-mãe*) and water, stuffed with streaky pork (*entremeada*), dried meat sausage (*chouriça*) and onion.	North region
Bola de Lamego (*Bola de Carne de Lamego*; *Bola de Bacalhau de Lamego*; *Bola de Sardinha de Lamego*)	Wheat	Rectangle shape, with a weight of about 850 g. Wheat, salt, baker's yeast (or mother-dough), water and butter, lard or olive oil, stuffed with meats, codfish or sardines.	North region
Bolo de Farinha de Milho com Carne	Maize, rye, wheat	Flat base and round shape. Maize, rye and wheat (3/1/1), salt, mother-dough (*massa-mãe*) and water, stuffed with various pork meats, for instance dried smoked sausages (*salpicão*), chorizo (*chouriça*), smoked bacon (*toucinho defumado*), smoked ham (*presunto*).	North region
Bolo do Caco	Wheat	Round and flat bread shape, with 15 and 20 cm in diameter and 2 to 3 cm high, soft texture, baked in a tile fragment (*caco*) or clay skillet. Wheat, salt, starter (or mother-dough) and water. Long kneading, short fermentation and short baking.	Madeira archipelago
Bolo Folar da Guarda	Wheat	Folar cake. Long shape, with 0.5 to 2 kg weight. Wheat, salt, baker's yeast (or mother-dough), water, eggs, brandy and olive oil.	District of Guarda. Central region
Broa (*Broa de milho*; *Pão de milho*)	Maize, rye	Circular to ellipsoidal shape, round top and a flat basis, weight between 1 and 3.5 kg but can have *ca*. 5 kg. Bread made of regional maize and rye flours with varying proportions of maize and rye flours (from 50 to 85% of regional maize flour), salt, mother-dough (*massa-mãe*), water.	North region
Broa à Lavrador	Maize, rye, wheat	Similar characteristics as *broa*.	Municipality of São João da Madeira, Santa Maria da Feira, Oliveira de Azeméis, *etc*. District of Aveiro. North region
Broa de Avanca	Maize, rye	Similar characteristics as *broa,* weight between 1 and 5 kg, yellow corn flour. Yellow maize.	Municipality of Avanca. District of Aveiro

Broa de Avintes	Maize, rye, Malt	Two typical sizes and shapes: ellipsoidal, a round top and a flat basis, 8-9 kg weight, about 30 cm diameter and 15 cm height; and cylindrical, a round top and a flat basis, 1 kg weight, about 10 cm diameter and 15 cm height. White maize, rye, malt, salt, mother-dough (*massa mãe*), water.	Municipality of Avintes. District of Porto. North region
Broa de Milho da Beira Alta	Maize, rye	Similar characteristics as *broa*. Circular shape, a round top and a flat basis. Two typical sizes: 12-15 cm diameter and 800 g weight; and 10-12 cm diameter and 400 g weight.	Beira Alta region. Central region
Broa de Milho do Vale do Sousa	Maize, rye	Similar characteristics as *broa*. Circular shape, a round top and a flat basis.	Vale do Sousa sub-region. North region
Broa de Milho e Centeio	Maize, rye, Wheat	Similar characteristics as *broa*.	Between Douro and Tâmega sub-region. North region
Broa de Vil Moinhos	Maize, whole rye	Similar characteristics as *broa*. Maize of high granularity.	District of Viseu. Central region
Broa Mimosa do Boco	Maize, rye	Similar characteristics as *broa*.	Municipality of Vagos. District of Aveiro. North region
Caralhotas de Almeirim	Wheat	Round shape, about 15 cm diameter. Brittle and crunchy crust, brownish color and rustic crumb. Wheat, salt, mother-dough (*massa-mãe*) and water.	Municipality of Almeirim. District of Santarém. Central region
Fogaça da Feira	Wheat	Sweet bread with a light flavor and aroma of lemon and cinnamon, brownish hue and a conical shape with four nozzles at the top (representing the towers of Feira Castle). Loose and light dough, slightly yellowish in color, with small holes and crispy outside.	Municipality of Santa Maria da Feira. District of Aveiro. North region
Folar de Trás-os-Montes	Wheat	Folar cake. Rectangular or round shape, 0.5-3 kg weight. It is only made at Easter. Wheat, salt, mother-dough (*massa-mãe*), water, eggs, oil and butter, stuffed with smoked pork meats of ham (*presunto*), dried smoked sausage (*salpicão*) and bacon (*toucinho*).	Trás-os Montes region. North region
Folar de Valpaços	Wheat	Folar cake. Rectangular shape. Wheat, salt, mother-dough (*massa-mãe*), water, with eggs, olive oil and vegetable margarine and/or lard, stuffed with fat meat pork and/or salted and dried streaky (*entremeada*), salted and dried pork, dried smoked pork sausages (*salpicão* and *linguíça*), smoke-cured or naturally cured pork ham (*presunto*) and/or smoked pork shoulder (*pá de porco*).	Municipality of Valpaços. Trás-os Montes region. North region
Padas do Vale de Ílhavo	Wheat	Small wheat breads, made up of two small units overlapping at one end. Golden and soft crust. Wheat, salt, mother-dough (*massa-mãe*) and water.	Ílhavo valley. District of Aveiro. North region
Pão com Chouriço (*Pão Caseiro do Marco*)	Wheat	Bread in the shape of a small yellowish bagel, with an aroma of smoked meat and a fluffy texture. Wheat, salt, mother-dough (*massa-mãe*) and water, stuffed with chorizo (*chouriço*)	Municipality of Marco de Canaveses. North region
Pão de Alfarroba	Wheat, Carob	Circular, round top and a flat basis shape, with a flavor reminiscent of chocolate, very dark in color and sweet in flavor. Wheat (85%), Carob (15%), salt, baker's yeast (or mother-dough) and water.	Algarve region. South region
Pão de Casa (*Pão Regional*; *Pão de Trigo da Terra*; *Pão de Família*)	Wheat, Sweet potato	Circular to ellipsoidal format, a round top and a flat basis, around 1 kg weight. Wheat (75%), sweet potato (17%), salt, mother-dough (*massa-mãe*) and water.	Madeira archipelago

Pão de Centeio da Guarda	Rye	Ellipsoidal shape, a round top and a flat basis, with a relatively crispy and cracked crust. Three typical sizes: small, 10-12 cm diameter and 400 g weight; medium, 13-15 cm diameter and 800g weight; and large, 16-18 cm diameter and 1200 g weight. Rye, salt, baker's yeast (or mother-dough), water.	District of Guarda. Central region
Pão de Centeio de Barroso	Rye	Circular to ellipsoidal shape, round top and a flat basis, 3 kg weight, dark color. Rye, salt, mother-dough (*massa-mãe*) and water.	Barroso sub-region. North region
Pão de Centeio de Castro Laboreiro	Rye, Wheat or Maize	Circular shape, a round top and a flat basis, 3-4 kg weight. Dark in color, acidic, not very moist, with a thin crust. Rye, wheat or maize, mother-dough (*massa-mãe*), salt and water.	Castro Laboreiro sub-region. Central region
Pão de Centeio do Sabugueiro	Rye, Wheat	Circular shape, a round top and a flat basis, 40-50 cm diameter 1-1.5 kg weight. Interior appearance is porous with a relatively crispy crust. Dark pearl-white color. Rye (> 60%) and wheat, mother-dough (*massa-mãe*), salt and water.	Sabugueiro sub-region. Central region
Pão de Cornos (*Pão de azeite*)	Wheat	Shape of a half-moon. Rough texture, light brown color, olive oil flavor and odor. Wheat, olive oil, mother-dough (*massa-mãe*), salt and water.	Municipality of Vagos. District of Aveiro. North region
Pão de Escalhão	Rye, Wheat	Circular shape, a round top and a flat basis, 40-50 cm diameter 1 kg weight. Interior appearance is dry and porous, with a relatively crispy and cracked crust. Pearl-white color on the inside and light brown on the outside. Rye, wheat, baker's yeast (or mother-dough), salt and water.	Central region
Pão de Mafra (*Pão saloio*)	Wheat	Round, long or ball shape. Small size. Soft texture to the touch, both on the crust and inside. Normally, crumb with little density, large holes or alveoli. High humidity (32-40%) and low acidity. Low amounts of starter. Wheat, baker's yeast (or mother-dough), salt and water.	Municipality of Mafra. Central region
Pão de Padronelo (*Pão de Ovelhinha*)	Rye, Wheat	Family bread as its shape makes it easy to divide into four parts, rectangular shape, 10-15 cm diagonally, 5 cm height, 150 g weight. Yellowish-brown color and firm and smooth consistency. Rye, wheat, mother-dough (*massa-mãe*), salt and water.	Municipality of Amarante. North region
Pão de Trigo do Alentejo (*Pão Alentejano*)	Wheat	Forehead bread shape (higher at one end, resembling a fold), 1-1.5 kg weight. Light brown crust and a whitish crumb. Wheat, mother-dough (*massa-mãe*), salt and water.	Alentejo. South region
Pão de Trigo em "Padas"	Wheat	Rustic-shaped bread, made up of two small units (about 50 g) overlapping at one end. Three variants: "white" (type 55 flour); "clean" (type 65 flour); and "dark" (type 80 flour). Floured crust, typically from a very soft dough, and irregular crumb. Sharp flavor and slightly acidic. Long baking, giving a characteristic crispy crust. Wheat, mother-dough (*massa-mãe*), salt and water.	District of Aveiro. North region
Pão Doce	Wheat	Circular shape, a round top and a flat basis. Sweet bread, with a soft texture, and lemon and cinnamon flavor. Characteristic crust from baking in a wood oven. Wheat, olive oil or butter, mother-dough (*massa-mãe*), salt and water, eggs, sugar, lemon zest and cinnamon. Egg whites should only be half the amount of eggs.	Municipality of Vagos. District of Aveiro. North region
Pão Podre	Wheat	Ring shape, a round top and a flat basis, 1 kg weight. Sweet flavor and brownish color. Wheat, baker's yeast (or mother-dough), salt and water, sugar, eggs and cinnamon.	Municipality of Marco de Canaveses. North region
Pão Santoro de Pêga	Wheat	Flat and elongated shape, with rounded ends, slightly concave in the longitudinal direction, approximately 40-50 cm long and 2 cm high, which can reach 4 cm high at the ends, 250-350 g weight. It is slightly concave in the longitudinal direction and has a relatively crispy crust, with a floury and fatty appearance. It has a dark pearl-white color. Relatively crispy crust, with a floury and fatty appearance. Dark pearl-	Municipality of Sabugal. District of Guarda. Central region

		white color. Wheat, baker's yeast (or mother-dough), salt and water, sugar, brandy (*aguardente*) and local olive oil.	
Pão Sovado (*Pão Arregueifado*)	Wheat	Different shapes and weights depending on the regions: B*icas* (spouts) – small units finished in a spout with a longitudinal cut; *Regueifinhas* – units with about 0.5 kg of dough in the shape of a snail; *Regueifas* – two elongated pieces with two palms long, braided, with the final shape of a braided ring and the surface decorated with shaped small rings and leaves; *Calo* – elongated mass whose ends are joined in the middle and creased with the hand in a cleaver; and *Redondo* (round) – flattened dough on the surface of which a square is made. Smooth crust and a very closed and regular crumb. Hard dough that needs to be bound by compressing with kneading cylinders. Variants: *Pão redondo* (round bread) (in the border areas of Beira Alta region); and *Pão de Calo* (in the Baixo Alentejo and Algarve regions). Wheat, mother-dough (*massa-mãe*), salt and water (only 50% of the typical amount of water used).	North region. Other regions
Pãozinho (*Carcaça*; *Bijou*; *Molete*; *Papo-seco*; *Rosca*; *Viana*)	Wheat	Small bread with different shapes depending on the regions: *Carcaça* (carcass) (Lisbon and Tejo valley) – slightly elongated, creased in the middle, with a very fluffy crumb and crispy crust; *Bijou* or *Molete* (between Douro and Minho regions) – round bread, opened where the cut was made before baking; *Papo-seco* (Ribatejo, Alentejo and Southwest regions) – slightly elongated, creased in the middle and ending in small heads; *Rosca* (Lisbon region) – elongated, made from two twisted strips of dough, with a rough and crispy crust; Viana (Lisbon region) – very fluffy round bread. Wheat, baker's yeast, salt and water.	Lisboa and Tejo Valley. Central region. Other regions

Broa de milho is widely produced at farmer level in the Northern Portugal and it is the most scientifically studied. Other types of *broas* made of maize and rye flours (sometimes with some wheat flour) exist mainly in North and Center Portugal, for instance *Broa à Lavrador*, *Broa de Avanca*, *Broa de Milho da Beira Alta*, *Broa de Milho e Centeio*, *Broa de Vil Moinhos*. *Broa de Avintes*, in Porto (Portugal) region, is one of the most known *broa* made of maize, rye and malt flours, and produced with traditional processes but at semi-industrial scale as a result of its commercial success (Novotni et al., 2021; Rocha, 2011; Rocha *et al*., 2023).

The general breadmaking process of Portuguese sourdough *broa* (**Figure 1**) is described in several publications (Rocha, 2011; Rocha and Malcata, 1999, 2012, 2016a, 2016b; Rocha *et al*., 2003, 2006, 2010a, 2010b, 2011, 2012a, 2012b, 2023) and varies according to the farmers and geographical locations. Briefly, traditional *broa* (**Figures 1g** and **1h**) is a type-I sourdough bread made of maize and rye flours by households and small farmers and following ancient procedures empirically transmitted between generations. Regional maize is still being milled in watermills by some farmers in the rural villages but it is getting rarer. The percentage of maize and rye flours for sourdough *broa* also varies according to the producer, and may ranges between 50 and 85% of maize flour. *Broa* may have different formats, from circular to ellipsoidal, with a flat basis and a round top, and with an average weight of approximately 1.5 kg, but also varying between *ca*. 1 and 5 kg. Sourdough *broa* crust may have a thickness of approximately 1-2 cm. *Broa* is usually produced every 15 days in winter and weekly during summer. The sourdough *broa* fermentation is usually undertaken in two steps, the so-called first and second fermentation. In the day before baking it is prepared the sourdough, sometimes known as *crescente* (crescent): maize flour is sieved into a wooden kneader (to separate the bran from flour) and manually kneaded for 10-20 minutes, with salted warm water and rye flour. Rye flour is usually purchased in the local market but can also be produced by some farmers of certain North regions. Afterwards, mother-dough is added and mixed – *i.e*. a small portion of mature sourdough from the previous baking batch and acting as the microbial starter culture, that is a reservoir of adventitious microorganisms. This mixture is let to ferment usually overnight, at room temperature, but may vary as much as from 4.5 to 28 h. Early in the next morning, the largest amounts of sieved maize and rye flours are used for the breadmaking. Firstly, regional maize flour is scalded and kneaded with salted warm water, usually with a wooden shovel due to the high temperatures of the water, then rye flour and the sourdough from the previous day (first fermentation) is added and manually kneaded. This laborious and physically demanding kneading process usually takes 30-40 min, after which the baking dough is let to ferment. This second fermentation, undertaken at room temperature, also varies between householders from 1.5 to 3 h. When baking dough starts opening, the bakers know that the fermentation was successful and ready. During the second fermentation, the clay wall oven is heated with firewood and temperatures may reach approximately 250 ºC. After the second fermentation, sourdough is molded with a wooden bowl (called "*gamelo*" or "*tigelão*") and, then, is taken into the oven usually with a round or rectangular wooden shovel. The clay oven is closed and sealed and *broas* are let to bake during approximately 2 hours.

One of the new trends in bakery production is the rediscovery of wheat landraces with particular nutritional and/or technological properties. Sicilian ancient durum wheats have been reported to have a different impact on the overall quality of bakery goods. In particular, Majorca flour showed the highest amount of glutenin, compared to other Sicilian durum wheat landraces, such as Margherito, Perciasacchi, Russello and Timilia, that improved the gluten strength and the technological properties of the dough (Visioli et al., 2021). Among the ingredients, water has also been reported to play a pivotal role in the traditional features of bakery goods since several correlations were found between sourdough microbiota and water features (Minervini et al., 2019). However, in spite of some differences in the technological aptitude of the flours and the production process, almost all traditional typical Italian breads are obtained by using sourdough as the natural starter, thus underlying the contribution of the microorganisms to the typicity of the final products (Minervini, Di Cagno, et al., 2012).

In the last years, some biotechnological innovations have been reported in sourdough fermentation, mainly including the employment of innovative strains in order to improve the overall quality of these foodstuffs (*i.e.* technological, nutritional, functional sensorial and safety-related features), thus reducing the risk associated to spontaneous fermentation (Capozzi et al., 2017; Gobbetti et al., 2019). For example, the incidence of intolerance to baker's yeast is increasing in the Western population, mainly due to some cell wall components of *Saccharomyces cerevisiae* that have been recognized as antigens in individuals with chronic inflammatory bowel disease (IBD). Therefore, a combination of unconventional yeast *Zymomonas mobilis*, isolated from fermented agave sap and *Fr. sanfranciscensis* has been proposed as a driver of innovation in the area of yeast-free leavened products (Musatti et al., 2016; Picozzi et al., 2022). In a similar way, a strain of *Leuconostoc citreum* inoculated in a liquid sourdough (type-II sourdough) has been employed for the production of a yeast-free and salt-free typical Apulian bread, known as *puccia* (De Bellis et al., 2019). In particular, salt was eliminated because it negatively impacted on the viability of *L. citreum*. However, the sensory quality of the bread was acceptable and the suitability of this strain as a microbial starter was confirmed at the pilot scale in an industrial bakery (De Bellis et al., 2019). A similar protocol was applied to produce a yeast-free *focaccia* with reduced salt content. In particular, salt was replaced with seawater, thus introducing a technological innovation for producing a traditional baked good with added beneficial health effects due to the lowest predicted glycemic index (GI), salt reduction and magnesium cation (Mg^{2+}) fortification (De Bellis et al., 2020). According to the market's current trends, consumers are increasingly accepting bakery products with lower fat content. In this framework, the employment of exopolysaccharide (EPS)-producing strains of *Weissella cibaria* could be an attractive biotechnological solution to improve the rheological properties and substitute fat replacers (Bavaro et al., 2021). It is well-known that microbial dextrans increase the final volume, the softness of the crumb and the product's shelf-life, thus obtaining a special interest for application to the production of gluten-free baked goods (Ramos et al., 2021). On the other hand, dextrans synthesized by *W. cibaria* have been reported to be bioactive compounds with immuno-stimulants and anti-inflammatory properties, supporting their ability to improve the functionality of bakery products (Zarour et al., 2017). Thus, over-producing riboflavin strains of *W. cibaria* have been proposed to obtain multifunctional breads biofortified with both vitamin B_2 and dextrans (Hernández-Alcántara et al., 2022). Recently, it has been reported that sourdough fermentation encouraged the conversion of inorganic selenium into organic selenium compounds resulting in a high content of bioaccessible selenium, suggesting that selenium-rich flours could be used for the production of a functional *piadina*, a typical flatbread of Emilia Romagna (Italy) (Di Nunzio et al., 2018). Interestingly, a functional traditional sourdough, the so-called "*ciabatta*" bread, obtained by fermentation with selected strains of sourdough supplemented with insect powders, opens new perspectives in the field of novel foods applications and in the production of biofortification in the protein content (Gaglio et al., 2021).

(a)

(b)

(c)

(d)

(e) (f)

(g) (h)

Figure 1. Household breadmaking of traditional Portuguese sourdough *broa*. (**a**) Sieving maize flour; (**b**) Scalding maize flour with warm water with salt, followed by the addition of rye flour and mother-dough ("*isco*") and manually mixing; (**c**) Manual kneading; (**d**) Smoothing the baking dough and letting it to ferment with the endogenous microbiota; (**e**) Heating the oven with firewood; (**f**) Molding sourdough and putting it into the warm oven, followed by closing and sealing the oven; (**g**) Sourdough *broas* in the oven after baking; (**h**) Sourdough *broa*. Courtesy of Dr. Jorge Miranda from In.Cubo – *Incubadora de iniciativas empresariais inovadores* [In.Cubo – Incubator of innovative business initiatives] (https://www.incubo.eu/, accessed in 2023-08-30).

3. Microbial diversity of traditional Italian and Portuguese sourdoughs

Southern Europe boasts a multiplicity of typical breads and sourdough-based traditional products whose microbial communities and dynamics have been extensively characterized (**Table 3**). Recently, the microbiota of Tuscan sourdough bread has been investigated by both culture-independent and dependent methods. Though a total of 68 yeasts and 96 LAB isolates were molecularly identified, bacteria belonged only to the species *Fr. sanfranciscensis,* while yeasts were identified as *Candida milleri* and only three isolates as *S. cerevisiae* were found. The relative composition and specific physiological characteristics of such microbiota can potentially affect the nutritional features of PDO Tuscan bread, as suggested by the functional characterization of the strains (Palla et al., 2017). A low microbial diversity was also found in sourdoughs from the Apulian region, which revealed the occurrence of *Lp. plantarum* and *S. cerevisiae* as the dominant LAB and yeasts, ranging their concentration between 1.7×10^5 and 6.5×10^8 colony-forming units (CFU)/g, or 7.7×10^5 and 2.5×10^7 CFU/g, respectively. In particular, the isolated LAB strains showed interesting antimicrobial activity, suggesting their potential exploitation to extend the shelf-life of sourdough bread (Arena et al., 2019). In agreement, 28 typical sourdoughs of Irpinia, a large area of the Campania region (Southern Italy), were submitted to molecular approaches revealing a high biodiversity in LAB community among the samples. The most abundant lactobacilli species belonged to *Lp. plantarum* (*ca*. 22% of total LAB isolates), *Fr. sanfranciscensis* (11%), *Companilactobacillus paralimentarius* (8%), and *Furfurilactobacillus rossiae* (6.5%), whereas LAB cocci include *Pediococcus pentosaceus* (9.5%), *Leuconostoc* spp. (7.8%), and *W. cibaria* (7.7%). However, most of the samples were characterized by the dominance of only one or two species, thus confirming the impact of the environment and sourdough fermentation on the development and persistence of few LAB better adapted to specific geographical, sourdough fermentation conditions and/or ecological niches (Reale et al., 2019). In another study, the microbiota of 19 Italian sourdoughs used for the manufacture of traditional breads allowed the identification, through a culture-dependent approach, of 20 LAB species, with a dominance of *Fr. sanfranciscensis*, *Lp. plantarum* and *Co. paralimentarius*, and four yeast species. Interestingly, *S. cerevisiae* was identified in 16 sourdoughs, while the yeasts *C. humilis*, *Kazachstania barnettii* and *Kazachstania exigua* were found as less represented species, thus indicating that differences in the microbial composition could contribute to enhancing the typicality of traditional breads (Minervini, Di Cagno, et al., 2012). In consideration of the general occurrence of this species in traditional Italian sourdoughs, typing by combining conventional and multiplex RAPD–PCR profiles has been reported as an interesting tool to investigate the intra-specific variability among *Fr. sanfranciscensis* isolates (Venturi, Guerrini, Granchi, et al., 2012). However, some sourdoughs, such as Maiorca, typical from Sicilia region, showed a higher microbial variability. Indeed, the LAB *Lp. plantarum* and *Lev. brevis* dominated this ecosystem, while *S. cerevisiae* and *W. anomalus* were the most frequently isolated yeasts. In addition, *T. delbrueckii*, *Pichia kluyveri*, *Candida boidinii*, and *Candida diddensiae* were also detected (Pino et al., 2022). A polyphasic approach was used to determine the yeast microbiota associated with spontaneous sourdough fermentations in the production of twenty traditional wheat sourdough breads of the Abruzzo region (Italy). PCR-RFLP analysis identified 85% of the isolates as *S. cerevisiae*, being other dominant species *Candida milleri* (11%), *C. krusei* (2.5%) and *T. delbrueckii* (1%), thus providing an exhaustive overview of the yeast populations in sourdoughs from this region (Valmorri et al., 2010). A metagenetic analysis was performed on *Focaccia* obtained by yeast or LAB starter-mediated fermentation. Interestingly, samples made with baker's yeast showed a dominance of *Proteobacteria* (synonym *Pseudomonadota*, which is a major phylum of Gram-negative bacteria) (82% of the bacterial population), are associated with negative biochemical features of the dough. *Proteobacteria* population reduced to 43% when *L. citreum* was used as inoculum, indicating the ability of this starter to drive the fermentation, improving the microbiological quality of yeast-free bakery products (Ferrara et al., 2021). Indeed, such results underline the interest on using sourdough fermentation towards food safety.

Table 3. Microbial diversity in traditional sourdoughs and sourdoughs-based products in Italy and Portugal

Product	Geographical origin/Typical	Lactic Acid Bacteria (LAB)	Yeasts	Method of detection	Reference

	features of the product				
Cornetto di Matera	Bread - Matera (Basilicata, Southern Italy)	*Lp. plantarum* (49%) *L. mesenteroides* (17%) *Latilactobacillus curvatus* (15%) *Lactiplantibacillus paraplantarum* (12%) *W. cibaria* (5%) *Lactiplantibacillus pentosus* (2%).	---	SDS-PAGE	(Zotta *et al.*, 2008)
Tuscan bread	Bread - Tuscan	*Fr. sanfranciscensis* (100%)	*C. milleri* (95%) *S. cerevisiae* (5%)	ARDRA (LAB) RFLP (yeasts)	(Palla *et al.*, 2017)
Traditional sourdoughs	Nineteen typical sourdoughs for bread production – Different Regions of Italy	*Fr. sanfranciscensis* (28%) *Lp. plantarum* (16%) *Lp. paralimentarius* (14%)	*S. cerevisiae*, *C. humilis*, *K. barnettii*, *K. exigua*	RAPD-PCR	(Minervini *et al.*, 2012)
Maiorca sourdough	Sourdough from ancient durum wheat landraces -Sicily, Southern Italy	*Lp. plantarum* *Lev. brevis*	*S. cerevisiae* *W. anomalus* *T. delbrueckii*, *Pichia kluyveri*, *Candida boidinii*, *Candida diddensiae*	rep-PCR (bacteria) RFLP (yeasts)	(Pino *et al.*, 2022)
Traditional sourdoughs	Six typical sourdoughs for bread production - Apulia (Southern Italy)	*Lp. plantarum*	*S. cerevisiae*	ARDRA (LAB) RFLP (yeasts)	(Arena *et al.*, 2019)
Traditional sourdoughs	Twenty-eight typical sourdoughs for bread production - Irpinia (area of the Campania region, Southern Italy)	*Lp. plantarum* (22%) *Fr. sanfranciscensis* (11%) *Co. paralimentarius* (8%) *Fu. rossiae* (6.5%) *P. pentosaceus* (9.5%) *Leuconostoc* spp. (7.8%) *W. cibaria* (7.7%)	---	PCR-DGGE	(Reale *et al.*, 2019)
Traditional sourdoughs	Twenty typical sourdoughs for bread production - Abruzzo (central Italy)	---	*S. cerevisiae,* (85%) *C. milleri* (11%), *C. krusei* (2.5%), *T. delbrueckii* (1%)	PCR-RFLP, RAPD-PCR, PCR-DGGE	(Valmorri *et al.*, 2010)
Traditional sweet leavened baked goods	Eighteen sourdoughs for traditional Italian sweet leavened baked goods – Different Regions of Italy	*Fr. sanfranciscensis* (>80%) *Lp. plantarum* *L. citreum*	*S. cerevisiae* *C. humilis*	Pyrosequencing	(Lattanzi *et al.*, 2013)

Brioches and *cornetto*	Sweet baked goods – (Campania, Southern of Italy)	*Lactobacillus* spp. *Leuconostoc* spp. *Lactococcus* spp. *Fr. sanfranciscensis* *Lat. sakei* *W. groceries,* *S. thermophilus,*	*S. cerevisiae* *M. pulcherrima*	PCR-DGGE	(Palomba *et al.*, 2011)
Colomba	Easter sweet baked good - Italy	*Fr. sanfranciscensis*	*T. delbrueckii* *C. humilis* *S. cerevisiae* *K. marxianus*	NGS (MiSeq)	(Raimondi *et al.*, 2017)
Colomba	Easter sweet baked good - Italy	---	*C. milleri* *S. cerevisiae*	RAPD-PCR	(Vernocchi *et al.*, 2004)
Panettone	Christmas sweet cake -Northern of Italy	*Fr. sanfranciscensis* *Lev. brevis*	*C. humilis*	PCR-DGGE	(Garofalo *et al.*, 2008)
Panettone	Christmas sweet cake -Northern of Italy	*Fr. sanfranciscensis* *Lp. plantarum* *Fur. rossiae* *Len. parabuchneri* *L. mesenteroides* *L. citreum*	*Kazachstania humilis* *S. cerevisiae*	rep-PCR (bacteria) RFLP (yeasts)	(De Vero *et al.*, 2021)
Lagaccio *Panettone*	Dry biscuit – Genoa (Liguria Region, Italy) Christmas sweet cake -Northern of Italy	*Fr. sanfranciscensis*	*C. milleri*, *S. cerevisiae*	ARDRA (LAB) RFLP (yeasts)	(Venturi *et al.*, 2012)
Broa de milho	Northern (and center) Portugal	*Lev. brevis*, *Lactobacillus delbrueckii* ssp. *lactis*, *Lp. plantarum*, *Lat. curvatus*, *Lactobacillus delbrueckii* ssp. *delbrueckii*, *Leuconoctoc* spp., *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *Pediococcus* spp., *Gardnerella vaginalis*, *Streptococcus agalactiae*, *Streptococcus constellatus*, *Streptococcus equinus*	*Saccharomyces cerevisiae*, *Hansenula anomala*, *Kluyveromyces marxianus* var. *lactis*, *Pichia guilliermondii*, *Torulaspora delbrueckii*, *Pichia ohmeri*, *Pichia membranaefaciens*, *Issatchenkia orientalis*, *Pichia anomala*, *Lachancea kluyveri*, *Issatchenkia occidentalis*	Biochemical tests	(Novotni *et al.*, 2021; Rocha, 2011; Rocha and Malcata, 1999)

To retrieve their origin in sourdough, LAB population have been monitored from ear harvest until the first step of fermentation in order to clarify the relationship among wheat variety, geographical area of production and processing (Alfonzo et al., 2017). In agreement, differences in dominant LAB have also been reported during artisanal pasta-making manufactory located in Apulian Region (South Italy) (Russo et al., 2010).

Similar studies were performed to characterize the microbial ecosystems in sourdough employed for the production of sweet-leavened goods. A comprehensive study investigated the occurrence of LAB and yeasts by culture-dependent method and pyrosequencing in eighteen sweet sourdoughs. Viable cells were about 8.05 and 7.03 log CFU/g for LAB and yeasts, respectively. *Fr. sanfranciscensis* was identified in all the sourdoughs, except for *Panaredda* and *Torcolo di San Costanzo*, by using culture-dependent approaches. Intriguingly, *Fr. sanfranciscensis* was the only species detected in eleven sourdoughs and dominated, with at least 80% of the LAB population, traditional sourdoughs, such as *Buccellato di Lucca*, *Mbriagotto*, *Pandoro*, and *Nadalin*. Other LAB found with a relatively high frequency belonged to *Lp. plantarum* and *L. citreum*. Molecular approaches confirmed these results, and in a few cases, pyrosequencing complemented the culture-dependent methods, detecting *Fr. sanfranciscensis* also in *Panaredda* and *Torcolo di San Costanzo* sourdoughs. Among yeasts, *S. cerevisiae* was identified in all the sourdoughs, except for *Mbriagotto*, *Ciambella di Mosto* and *Pandolce Genovese*. These latter sourdoughs harbored strains of *C. humilis*, whereas five sourdoughs combined the presence of both yeast species (Lattanzi et al., 2013). Similarly, polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) analysis allowed to discover different levels of biodiversity in nine Italian sweet sourdoughs. Indeed, additional species than what detected by conventional culture-dependent methods were found in association with *S. cerevisiae*, including *Streptococcus thermophilus*, *Latilactobacillus sakei*, *Weissella groceries*, *F. sanfranciscensis* and *M. pulcherrima*, suggesting that typical microbial consortia could be selected for naturally fermented *brioche* and *cornetto* preparation (Palomba et al., 2011).

It has been reported that different technological parameters can impact on the final quality of the products (Novotni et al., 2021; Rocha, 2011). For example, a higher temperature employed to propagate sourdough for *Pandoro* production resulted in a lower pH that was associated to higher LAB counts (about 1 log CFU/g higher) but without affecting yeast concentrations (Montanari et al., 2014). A similar result has been found investigating two sweet bakery goods, namely *Lagaccio,* a typical Genoese dry biscuit, and *Panettone*, over a period of three years. In particular, stable microbial associations were characterized by *Fr. sanfranciscensis*, *C. milleri* and *S. cerevisiae* dominance. However, these two products exhibited differences in the ratio LAB/yeasts, probably ascribed to moderate refrigeration conditions in *Panettone* manufacturing (Venturi, Guerrini, & Vincenzini, 2012). A sourdough for the industrial production of *Colomba* showed a small biodiversity in terms of both LAB and yeasts. Culture-dependent analyses identified *Fr. sanfranciscensis* and *T. delbrueckii*, as dominant bacterial and yeast species, respectively. These results were confirmed by metataxonomic analysis only for the bacterial communities, whereas *C. humilis* was the most abundant yeast. A low microbial biodiversity has been considered an advantage in terms of stable and easier propagation because responsible of more reproducible lots of *Colomba* (Raimondi et al., 2017). Eighty-six yeast isolates from the productive process of *Colomba* were characterized by phenotypic methods and identified by random amplified polymorphic DNA (RAPD)-PCR as *C. milleri* and *S. cerevisiae*, being *C. milleri* the dominant species in the sourdough. In particular, this maltose-negative yeast seems to lead strict cooperation with heterofermentative LAB that improve some technological and organoleptic features of the products (Vernocchi et al., 2004).

Similarly, LAB and yeast dynamics during the production of three varieties of *Panettone* were investigated by PCR-DGGE analysis. Although molecular fingerprinting provides evidence of the dominance of *Fr. sanfranciscensis*, *Lev. brevis* and *C. humilis*, a shift in the final stages of two of the production processes suggested an impact of some technological conditions on the dough microbiota (Garofalo et al., 2008). More recently, the molecular characterization of 77 isolates from two doughs for the production of *Panettone* revealed the presence of the dominant yeast *K. humilis*, while *S. cerevisiae* was found in only one sample. Among the LAB species, *Fr. sanfranciscensis* was the most abundant in both sourdoughs, with a lower occurrence of *Lp. plantarum*, *Fu. rossiae*, *Lentilactobacillus parabuchneri*, *Leuconostoc mesenteroides* and *L. citreum*. Moreover, microbial strains correlated with the volatile organic compounds synthesized during the processing suggested the employment of the best candidates in pure and/or mixed fermentation to contribute to the aromatic profile (De Vero et al., 2021).

Rocha and Malcata (1999) undertook a culture-dependent taxonomic identification of several groups of bacteria and yeasts in maize and rye flours and respective sourdough used for the manufacture of traditional sourdough *broa*. The novelty of this work lay in the study of this Portuguese traditional specialty for the first time and the broad spectrum of classes of bacteria scrutinized. Overall, it was identified 375 microbial isolates but only the results of sourdough are here described and analyzed. Lactic acid bacteria and yeasts were the dominant species in sourdough. Moreover, it was unfolded a decrease in the all the microbial species diversity from maize and rye flours to the sourdough, as a result of the fermentation that drives a spontaneous competitive selection of the microorganisms existing in the raw-materials.

Facultative anaerobic Gram-negative rod-shaped Enterobacteriaceae were identified in maize and rye flours but not in sourdough. Aerobic Gram-negative rod-shaped Pseudomonadaceae were detected in sourdough, with *Sphingobacterium paucimobilis*, *Pseudomonas cepacia* and *Pseudomonas* spp. being the most common identified, in addition to other identified species, chiefly *Pseudomonas stutzeri*, *Xanthomonas maltophilia*, *Chryseomonas luteola* and *Agrobacterium radiobacter*. The Gram-negative rod *Achromobacter* spp. was also detected. Regarding the endospore-forming Gram-positive rods, it was identified several species belonging to the Bacillaceae family: *Bacillus pumilus*, *Bacillus cereus*, *Bacillus circulans*, *Bacillus laterosporus*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus mycoides*, *Bacillus polymyxa*, *Bacillus stearothermophilus*, *Bacillus badius* and *Bacillus brevis*. Furthermore, a large diversity of Gram-positive catalase-positive cocci was detected in maize and rye flours but such a diversity decreased in sourdough. Lastly, it was detected *Micrococcus* spp. and *Micrococcus kristinae*, belonging to the Micrococcaceae family, and *Staphylococcus lentus*, *Staphylococcus xylosus*, *Staphylococcus chromogenes*, *Staphylococcus schleiferi*, *Staphylococcus sciuri* and *Staphylococcus aureus*, belonging to the Staphylococcaceae family (Rocha and Malcata, 1999).

In respect to the regular nonsporing Gram-positive rods belonging to the family of Lactobacillaceae, the main homofermentative or facultative heterofermentative *Lactobacillus* species identified were *Lev. brevis*, *Lactobacillus delbrueckii* ssp. *lactis*, *Lp. plantarum*, *Lat. curvatus* and *Lactobacillus delbrueckii* ssp. *delbrueckii*. Interesting to note that other lactobacilli species were found in maize and rye flours, *viz*. *Lactobacillus acidophilus*, *Lacticaseibacillus paracasei* ssp. *paracasei* and *Lev. brevis*. In addition, the nonsporing Gram-positive rod *Listeria* spp. was also detected. Moreover, Gram-positive catalase-negative cocci belonging to lactic acid bacteria were also identified in maize and rye flours and sourdough. Regarding the sourdough, the identified LAB species encompassed *Leuconoctoc* spp., *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *Pediococcus* spp., *Gardnerella vaginalis*, *Streptococcus agalactiae*, *Streptococcus constellatus* and *Streptococcus equinus* (Rocha and Malcata, 1999).

In respect to yeasts, Rocha and Malcata (1999) identified species belonging to the Ascosporogenous and imperfect yeast groups. In sourdough, the main identified species were *Saccharomyces cerevisiae* and *Hansenula anomala*, both belonging to the family Saccharomycetaceae. Other yeasts were the Cryptococcaceae *Kluyveromyces marxianus* var. *lactis*, the Cryptococcaceae *Pichia guilliermondii* and *Torulaspora delbrueckii*, and the Saccharomycetaceae *Pichia ohmeri*.

Almeida and Pais (1996a, 1996b) characterized the yeast population in 33 sourdoughs for *broa* production using culture-dependent techniques. *Saccharomyces cerevisiae* was the species mostly identified, followed by *Torulaspora delbrueckii*, *Pichia membranaefaciens* and *Issatchenkia orientalis* (also known as *Pichia kudriavzevii* or *Candida krusei*). Other identified species were *Pichia anomala*, *Lachancea kluyveri* (a budding yeast related to *Saccharomyces cerevisiae*), *Issatchenkia occidentalis* and *Kluyveromyces marxianus* (Almeida and Pais, 1996a). *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* isolated from *broa* sourdoughs were further subjected to studies on the leavening ability and freeze tolerance (Almeida and Pais, 1996b) and, more recently, *Torulaspora delbrueckii* has been intensely studied by Alves-Araújo *et al*. (2005a, 2005b, 2007) and Pacheco *et al*. (2020).

Aiming at characterizing the microbiota of maize and rye flours and respective *broa* sourdough and the effect of spontaneous sourdough fermentation, by comparing the microbiota of flours and respective sourdoughs, Rocha and Malcata (2012) made a comprehensive study on the cell viable counts with samples of maize and rye flours and sourdough provided by 14 farmers and households from different regions and sub-regions from the Northern Portugal and in two different time periods of the year (Autumn-Winter and Spring-Summer). The vegetative and (mesophilic and thermophilic) spore viable counts were undertaken for a large spectrum of microorganisms, encompassing total, mesophilic and thermophilic microorganisms, yeasts and molds (filamentous fungi), Gram-negative rods, endospore-forming and nonsporing Gram-positive rods, and catalase-positive and catalase-negative Gram-positive cocci. A total of 20 different general, selective and differential culture media were used and involved 26 different incubation conditions. The results unfolded no discrimination of the microbiological viable counts among samples from different regions or seasons but spontaneous sourdough fermentation represented a key-factor affecting the profile of microbiological traits of the samples, that is it played a major impact in the spontaneous selection of microorganisms initially found in maize and rye cereals, chiefly the viable counts of yeasts, lactobacilli, streptococci, lactococci, enterococci and leuconostocs increased and, simultaneously, the viable counts of molds, Enterobacteriaceae, Pseudomonadaceae, staphylococci and micrococci decreased. Such results highlighted the importance of cereals as the main contributor to the microbial diversity found in sourdough, as well as the role of spontaneous sourdough fermentation towards the prevalence of desired microorganisms in the sourdough matrix via complex interactions, such as competition and synergism, but other interactions may exist such as symbiosis, neutralism, commensalism, mutualism, protocooperation, parasitism, amensalism, antagonism, *etc*.

The results in this study from Rocha and Malcata (2012) outlined that general total viable counts, grown on tryptone soy agar (TSA) increased with sourdough fermentation, *i.e*. from cereal flours to sourdough, whereas total thermophilic viable counts were found in much less concentrations and were similar in the three type of studied food matrixes (maize and rye flours, and sourdoughs).

Facultative anaerobic Gram-negative rods were incubated in violet red bile dextrose agar (VRBDA) and MacConkey agar, whereas *Pseudomonas* agar base (PAB) was used for the aerobic Gram-negative rods. VRBDA is indicated for the growth of Enterobacteriaceae, and MacConkey for *Salmonella*, *Shigella*, *Yersinia* and others, as well as for coliform bacteria. The undesirable presumptive viable counts of facultative anaerobic and aerobic Gram-negative rods decreased from flours to sourdough. The average number of viable microorganisms in flours growing on VRBDA, PAB and MacConkey were, respectively, 5.7-6.0, 6.2-6.6 and 5.8-6.4 log (CFU/g) in flours, whereas in sourdoughs were only 1.4, 1.5 and 1.2 log (CFU/g), respectively (Rocha and Malcata, 2012).

Moreover, the viable counts of presumptive endospore-forming Gram-positive rods grown on *Bacillus cereus* medium (BCM) showed that *Bacillus* species are likely to find adequate growth conditions in sourdoughs and, thus, they persist after sourdough fermentation. *Bacillus* are linked with food spoilage and harmful effects on human health. The average number of vegetative cell forms grown on BCM was 6.1-6.6 log (CFU/g), whereas spore forms were 1.9-2.7 log (CFU/g). Although spores from Gram-positive rods may withstand the high baking temperatures, the lower of pH as a result of lactic acid bacteria fermentation may be able to inhibit spore germination (Rocha and Malcata, 2012). Moreover, Baird-Parker medium base (BPM) was used to enumerate the presumptive Gram-positive catalase-positive cocci *Staphylococcus* and *Micrococcus*. The results showed a significative reduction of these microorganisms from flours to sourdoughs, in average from 3.6-5.2 to 2.5 log (CFU/g) (Rocha and Malcata, 2012).

The regular nonsporing Gram-positive rods were incubated on de Man, Rogosa and Sharp agar (MRS); Gram-positive catalase-negative cocci belonging to LAB, such as *Pediococcus* and *Leuconostoc*, also grow on this culture medium. As a result of the fermentation, sourdough samples had higher viable counts than in cereal flours. The average viable counts were 5.4-5.8 log (CFU/g) in flours and 8.5 log (CFU/g) in sourdough (Rocha and Malcata, 2012).

LAB Gram-positive catalase-negative cocci were studied in several specific culture media, chiefly M17 agar, lactic streak agar (LSA), Kenner faecal streptococcal agar (KFS), kanamycin esculin azide agar (KEAA) and Mayeux, Sandine and Elliker agar (MSE). The presumptive *Streptococcus*, belonging to lactic acid bacteria (particularly, the homofermentative *Lactococcus*) obtained on M17 and LSA revealed similar or higher viable counts on sourdoughs when comparing to cereal flours, ranging from 6.5 and 7.8 log (CFU/g), respectively. The presumptive *Streptococcus* (particularly, the homofermentative *Enterocococcus*) viable counts were attained by using the KFS and KEAA culture media, and the results unfolded their increase after fermentation. The average viable counts on these two culture media were 3.2-4.3 in flours and 6.3-6.5 in sourdoughs. The average presumptive *Leuconostotc* viable counts obtained on MSE were 4.5-5.5 in flours and 6.1 in sourdoughs. Therefore, the LAB Gram-positive catalase-negative cocci viable counts showed that these microorganisms are present in the sourdoughs, which may play important roles when it comes slow sourdough fermentations (Rocha and Malcata, 2012).

Unlike mold viable counts, yeast viable counts were generally higher in sourdough than in flours – thus, indicating the importance of sourdough fermentation towards the reduction of spoilage microorganisms and subsequent extension of the shelf-life of sourdough breads. Yeasts and molds were inocculated in two different culture media and under different incubation conditions, *viz*. yeast extract dextrose chroramphenicol agar (YEDCA) and Rose-Bengal chloramphenicol agar base (RBCAB). The average number of yeasts were 3.6-4.1 log (CFU/g) in flours and 7.2-8.1 log (CFU/g) in sourdough, whereas the average of molds were 4.1-6.2 log (CFU/g) in flours and 0.2-2.1 log (CFU/g) in sourdough (Rocha and Malcata, 2012). Therefore, presumptive acid-tolerant yeasts and *Lactobacillus* dominated in sourdoughs after the overnight first fermentation, reaching an average value of 8.1 log (CFU/g), followed by the LAB Gram-positive catalase-negative cocci.

Overall, the study conducted by Rocha and Malcata (2012) pointed out for the importance of fermentation towards the predominant microbiota found in sourdoughs and their distinction from the microbiota found in the main raw-materials, the cereal flours. The discrimination of microbiological profiles of maize and rye flours and sourdoughs between regions and seasons was not observed, indeed. The process of sourdough fermentation employed by different local farmers and householders plays a major impact on the microbiological profile of sourdoughs and not allows the discrimination of microbiology of sourdoughs between geographical regions (Rocha and Malcata, 2012). The process of manufacturing traditional sourdough seems to play a major impact, *i.e.,* the exogenous factors like fermentation time and temperature, dough yield, number and time of back slopping steps, percentage and time of the mother-dough used as inoculum. Other important exogenous factors also encompasses, for instance, milling, sifting and kneading steps, sodium chloride addition and baking time and temperature. The endogenous factors in breadmaking also affect the final sourdoughs and sourdough breads, which are associated to the type and quality of the flours (for example, enzyme activity, buffering capacity, and composition of carbohydrate, peptide, amino acid and phenolic compounds) (Novotni et al., 2021; Rocha, 2011). However, the processing factors seems to have a major impact on the metabolic activity of sourdough microorganisms and to constitute the major reason for the variability of the microbiota found in different sourdoughs, thus making unfeasible their microbial discrimination between geographical regions or seasons. As previously described, among different producers the first fermentation of *broa* sourdough can be undertaken from 4.5 to 28 h, whereas the second fermentation can take 1.5-3 h, the mother-dough can be kept at room temperature or under refrigeration in a time variable, the ratio maize/rye flour varies, the frequency of *broa* breadmaking also varies significantly and holds a seasonality. These processing factors makes from each *broa* a unique baking product.

Having in mind the importance of fermentation for the final microbiological profile of *broa* sourdough, a new study was conducted by Rocha and Malcata (2016a) to ascertain the microbial dynamics of type-I sourdough throughout a long-term spontaneous fermentation of 39 days (sampling in days 0, 1, 2, 3, 7, 9, 14, 29 and 39). The same large spectra of culture media and incubation conditions used by Rocha and Malcata (2012) was employed for the enumeration of cell viable counts. The pH substantially dropped in the first 24 h of fermentation, playing a key-role towards the growth inhibition of undesired microorganisms – in particular the facultative anaerobic and aerobic Gram-negative rods (Enterobacteriaceae, Pseudomonadaceae and coliforms) since the endospore-forming Gram-positive rods (*Bacillus*) were ubiquitous throughout the spontaneous fermentation of *broa* sourdough. The pH found in wheat and rye sourdoughs are typically lower (*ca*. 3.6–3.9) than in *broa* sourdough, which is mainly due to the relatively short-time used by farmers in the second fermentation. Indeed, the average pH values in samples of *broa* sourdough and final *broa* were 4.2 and 5.2, respectively (Rocha and Malcata, 2016a).

General total viable counts on TSA increased in the first 48 h of fermentation, and after a small decline in number in day 3 the values stabilized until the end of 39 days of fermentation. Facultative anaerobic Gram-positive rods grown on VRBDA decreased significantly after 24 h of fermentation and disappeared by 72 h, whereas those grown on MacConley agar increased in the first 24 h of fermentation and were also vanished after 72 h of fermentation. Presumptive *Pseudomonas* counts on PAB increased substantially in the first 24 h and further decreased until being vanished after 7 days of fermentation (Rocha and Malcata, 2016a). These microbial dynamics shows that Gram-negative rods grows in the first 24 h of fermentation but are vanished by the pH drop and competition with the predominant LAB and yeasts. It also shows that increasing the time of the first fermentation of *bro*a sourdough could be positive to decrease the number of Gram-negative bacteria. Yet, the time of second fermentation might be prolonged.

Presumptive *Bacillus* grown on BCM increased rapidly in the first 24 h of fermentation and kept high count values during the entire fermentation period of 39 d, showing that a better attention for these microorganisms should be assumed. These endospore-forming Gram-positive rods grow in pH values ranging from 2 to 11, their spores survive to baking temperatures (although may be inhibited with low pH values), they cause ropiness in baking products and may be harmful to the consumers. Examples of species with this potential are *Bacillus cereus*, *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus licheniformis* and *Bacillus megaterium*. Moreover, the presumptive Gram-positive catalase-positive cocci *Saphylococcus* and *Micrococcus* grown on BPM appeared only in the first 24 h of fermentation being vanished thereafter (Rocha and Malcata, 2016a).

The regular nonsporing Gram-positive rods (*Lactobacillus*) grown on MRS increased significantly in the first 24 h of fermentation and kept in high values until day 9, after which decreased to values similar to the beginning of fermentation. Important to mention that such decrease was accompanied by the increase seen in yeasts. Presumptive *Lactobacillus* and LAB cocci were 10- to 100-fold higher than yeasts throughout a large time-period of 9 days of fermentation. Regarding the Gram-positive catalase-negative cocci grown on different culture media (M17, KFS, KEAA and MSE), it was disclosed a significant increase in the first 24 h of fermentation and the high values were somehow kept until day 9, after which they vanished, maybe due to the drying and acid conditions. Such a long time of viability indicates that these microorganisms play also an important role on sourdough fermentations (Rocha and Malcata, 2016a). Finally, yeast viable counts on YEDCA substantially increased in the first 24 h and from the day 9 on, showing their synergistic interaction with lactic acid bacteria and adaptation to relatively low pH values. Regarding molds grown on RBCAB, high viable counts were detected between days 7 and 14 of fermentation, and remained almost in the same value as at the beginning (Rocha and Malcata, 2016a).

From above it was unveiled that presumptive *Lactobacillus*, yeasts and *Bacillus* dominated at the end of the long-term fermentation, although present in small counts after kneading. *Staphylococcus* and Gram-negative rods, present in the dough after kneading, were inhibited during fermentation. After 24 h of fermentation, LAB Gram-positive catalase-negative cocci were present at significant levels. This long-term fermentation reached a quasi-stationary state. The results may suggest the use of 2 to 3 days of first fermentation of broa sourdough as a compromise between the development of endogenous desired microorganisms and disappearance of most of the microorganisms associated with food spoilage (Rocha and Malcata, 2016a).

Mother-dough – also known as sponge-dough, sour-ferment, mother-sponge or seed dough and known as “*isco*” (bait), “*massa-mãe*” (mother-dough), “*massa-azeda*” (sour dough) or “*crescente*” (crescent) in Northern Portugal (Rocha *et al*., 2023) – is the peace of leavened dough kept aside from baking batch to batch that constitutes a natural and spontaneous microbial starter culture for the traditional manufacture of sourdough *broa*. The addition of mother-dough accelerates spontaneous sourdough fermentations, and it is desirable to avoid deviations of quality between baking batches, which can be achieved by improving processing conditions, such as the storage time and amounts of mother-dough used. Mother-dough is usually stored at room temperature in the wooden kneader but nowadays is more often stored at refrigeration conditions (*ca*. 4 ºC). Based on this, Rocha and Malcata (2016b) led a study to ascertain the microbial dynamics of mother-dough during a storage time of 6 days (sampling in days 0, 1, 2 and 6) in a plastic bag under refrigeration conditions, using the same set of culture media and incubation conditions of Rocha and Malcata (2012, 2016a) and above described.

Total viable counts on TSA had a slight increase on days 2 and 6, indicating some slow activity of the adventitious microorganisms present in mother-dough. The average viable counts on TSA were 8.6 log (CFU/g) and small changes on these value (6% maximum variation) indicates the presence of a steady-state in the microbiota of mother-dough. Moreover, a steady average of pH value of 4.1 was measured in the mother-dough, predicting the low microbial activity present in mother-doughs stored at refrigeration conditions.

Viable counts of presumptive facultative anaerobic Gram-negative rods grown on VRBDA (Enterobacteriaceae) and MacConkey (*Salmonella*, *Shigella*, *Yersinia* and other coliforms) disclosed the absence of Enterobacteriaceae and an average value of 4.6 log (CFU/g) in MacConkey, demonstrating the effect of fermentation in reducing these undesired microorganisms. Unexpectedly, *Pseudomonas* viable counts on PAB (presumptive aerobic Gram-negative rods) were found in relative high concentrations, in average 7.0 log (CFU/g) (Rocha and Malcata, 2016a). As a matter of fact, it is expected the vanishment of Gram-negative rods, as stated in the study of long-term sourdough fermentation by Rocha and Malcata (2016b).

Spore-forming Gram-positive rods (*Bacillus*) grown on BCM were maintained at high concentrations in mother-dough over storage time, with a steady average value of 8.6 log (CFU/g) (Rocha and Malcata, 2016b). Such results are consistent with other works from the same authors (Rocha and

Malcata, 2012, 2016a). Furthermore, a steady and relatively low average value of Gram-positive catalase-positive cocci (*Stahylococcus* and *Micrococcus*), grown on BPM, of 4.3 log (CFU/g) was accounted in mother-dough (Rocha and Malcata, 2016b). In sourdough, the disappearance of these microorganisms is expected at *ca*. 48h (Rocha and Malcata, 2016a), thus their presence in mother-dough may indicate that longer fermentation of sourdough could be used.

Presumptive *Lactobacillus* in mother-dough (grown on MRS) were found in a steady average value of 8.8 log (CFU/g), which is 10-fold higher than yeast viable counts and shows their prevalence in sourdough fermentations. Gram-positive catalase-negative cocci LAB viable counts obtained on M17, KFS, KEAA and MSE culture media reached a steady grand-average of 8.4 log (CFU/g), making apparent their importance in sourdough fermentations in addition to *Lactobacillus* and indicating the need to have deeper studies for this group of microorganisms in sourdough (Rocha and Malcata, 2016b).

Sourdough LAB are usually sensitive to drying conditions. Therefore, keeping the mother-dough in plastic bags under refrigeration conditions seems to be a good strategy. LAB grow well at pH values ranging 4.0-4.5 but may be still active at pH values between 3.2 and 9.6. They are mesophilic microorganisms but some strains may grow between 5 and 45 ºC (Rocha and Malcata, 2016b). Acetic and lactic acids are the main contributors for sourdough total titratable acidity (TTA) (Rocha, 2011). The existence of both homofermentative and heterofermentative strains on sourdoughs are important because the first play important roles in obtaining a final bread with a good grain and elastic crumb, whereas the last is responsible for shorter and harder gluten, and is important to improve bread taste and aroma, as well as to indorse leavening (Rocha, 2011; Rocha and Malcata, 2016b). Organic acids produced by LAB have important anti-microbial properties, thus increasing, consequently, the shelf-life of sourdough bread). Fungicidal traits are firstly attributed to acetic acid (Novotni et al., 2021; Rocha and Malcata, 2016b). Conversely, lactic acid inhibits the growth of bacilli, which may survive to the high baking temperatures and are related to ropy spoilage of bread (Novotni *et al*., 2021; Rocha and Malcata, 2016b). Lactic acid is stronger than acetic acid in decreasing the pH but softer in flavor

(Rocha and Malcata, 2016b). Unlike lactic acid, acetic acid produced by heterofermentative LAB is a volatile with a sharp vinegar odor, thus producing different flavors and aromas in sourdough bread (Novotni et al., 2021; Rocha and Malcata, 2016b). Heterofermentative LAB releases acetic acid which may suppress yeast growth and such an effect may be avoided when slow acidification at low temperatures are employed (Novotni et al., 2021; Rocha and Malcata, 2016b).

In the same study of the microbial dynamics of mother-dough (Rocha and Malcata, 2016b), yeast (on YEDCA) and mold (on RBCAB) viable counts reached average values of 7.6 and 5.8 log (CFU/g), respectively. Unlike molds, yeasts tended to increase slightly (13% maximum variation, in both cases). The presence of yeasts and molds in mother-dough predict their capacity to maintain active at low temperatures, acidic conditions and high humidity. Additionally, the acidification of sourdough by LAB is slower at low temperatures, consequently favoring yeast activity – which optimal growth temperature is lower than those for lactobacilli. Conversely, low temperatures may reduce yeast activity because these conditions are favorable for the production of acetic acid by heterformentative LAB and yeasts are more sensitive to this type of organic acid. Furthermore, in the synergistic interactions between yeasts and LAB, yeasts produce growth factors, such as amino acids, peptides, vitamins, that favors LAB growth and, on the other side, LAB produce anti-microbial compounds, such as organic acids, hydrogen peroxide, carbon dioxide, ethanol, diacetyl, bacteriocins and bacteriocin-like inhibitory substances (BLIS), *etc*., that inhibits the growth of competitive microorganisms present in the mother-doughs and sourdoughs, including pathogenic and spoilage microorganisms present in cereal flours and baking doughs (Rocha and Malcata, 2016b). In general, all the studied microbial groups by Rocha and Malcata (2016b) displayed stability throughout storage under refrigeration conditions and suggests this process as suitable for the breadmaking of the Portuguese type-I sourdough *broa*.

Increasing the knowledge of the microbial communities during different sourdough fermentations paves the way for the selection of autochthonous beneficial strains further characterized by their technological properties. Accordingly, a large cohort of lactobacilli isolated from Italian sourdoughs have been screened for their proteolytic and peptidase activity in order to improve some functional properties of sourdough, such as antioxidant and anti-inflammatory activities (Galli et al., 2018). In another study, 41 LAB strains isolated from durum wheat sourdoughs for the production of *Cornetto di Matera* bread have been screened for their technological features (*i.e*. organic acids and exopolysaccharide production), as well their antimicrobial potential (Zotta et al., 2008). Similarly, one hundred LAB from Italian sourdoughs (Campania) were characterized for their tolerance to stress conditions commonly encountered within the production of leavened fermented bakery products, including the capability to face typical stress encountered during fermentation, such as acidic conditions, the occurrence of sodium chloride (NaCl) and ethanol, as well for some enzymatic activities (*i.e*. urease, amylase and proteolytic) or for the production of EPS. In particular, *Fr. sanfranciscensis*, *Fu. rossiae*, *Lev. brevis* and two strains of *Leuconostoc pseudomesentereoides* showed the highest survival to stress treatments and interesting technological properties (*i.e*. amino acid and exopolysaccharide production). Several strains exhibited high tolerance to the hardest stress conditions, suggesting their potential use for applications in bakery industry (Reale et al., 2020).

In order to determine if the bakery environment and equipment are effective sources of LAB and yeasts during the propagation of sourdough, their origin has been investigated in sourdough submitted to back-slopping steps by both culture-dependent and molecular approaches. Interestingly, *Fr. sanfranciscensis* was found to be the most abundant in equipment and sourdoughs. In contrast, *W. cibaria* showed higher adaptability in sourdough than in bakery equipment, suggesting that flours are the main origin of this species. *Lp. plantarum* persisted only in storage box, dough mixer and sourdough of two bakeries. *S. cerevisiae* was the dominant yeast in house and sourdough microbiotas, except in one bakery dominated by *K. exigua* (Minervini et al., 2015).

In order to investigate the contribution of the manufacturing process on the microbial composition of sourdough, seven mature type-I sourdoughs were back-slopped for 80 days at artisan bakery and laboratory levels under constant technology parameters. Interestingly, *S. cerevisiae* was no longer detectable in several sourdoughs during late laboratory propagation. Among LAB, *L. plantarum*, *L.*

sakei and *W. cibaria* dominated in only some sourdoughs back-slopped at artisan bakeries, while *Lat. citreum* seemed to be more persistent under laboratory conditions. In contrast, *Fr. sanfranciscensis* was found independently of the process. Similar studies could provide elucidation about the technological parameters leading to variations of the bacterial sourdough microbiota during the back-slopping allowing better control of industrial processes and standardization of high-quality baked goods (Minervini, Lattanzi, et al., 2012). A comparison between sourdoughs type-I and type-II obtained from stone-ground soft wheat revealed a similar stable microbiota characterized by a LAB concentration of about 9 log CFU/g, and the dominance of *Lev. brevis* and *Co. paralimentarius*. However, different volatile profiles were found among the experimental breads suggesting that the sourdough type significantly impacted the bread aroma (Cardinali et al., 2022).

4. Conclusion

Traditional sourdough breads using spontaneous slow fermentation by the complex microbiota naturally present in cereal flours and environment, and which resorts to the know-how empirically transferred from generation to generation, play important socio-economic, environmental and cultural roles. From an economic point of view, traditional sourdough breads and other baking goods (*e.g.* biscuits, crackers, pastry, pizza and pasta) represents an extra income for many local small farmers and householders. In the social standpoint, the presence of population in the rural areas contributes to combating desertification. Furthermore, sourdough breads are enjoying a growing popularity among consumers, since they are seen as convenient, nutritious, stable, natural and healthy food. In addition, sourdough-based products are highly appreciated for its distinct and unique flavors, aromas and texture, as well as for its nutritional attributes and extended shelf-life.

In terms of environment, the small local farmers constitutes important and active environmental protective elements, contributing to a sustainable agriculture and environmentally friendly agronomical practices, to the culture of authochthonous and ancestral cereal breeds, to the diversification of agriculture, and to the preservation of natural resources (soil, water, biodiversity) and plant genetic heritage. In fact, small farmers are often recognized to promote best agronomical practices to improve productivity, quality, safety and sustainability of food, and to mitigate and adapt to climate change. They are also of utmost importance concerning the territorial cohesion and are vital to create agricultural stains of discontinuity to prevent rural fires – which are increasing in number and dimension over the years due to the climatic change and desertification of rural areas, among other factors. Finally, in the cultural and historical standpoints, traditional bakery products and their ancient manufacturing protocols represents a heritage worth preserving with immeasurable value.

Therefore, it is important to save this immaterial cultural heritage and increase hygiene and safety standards of the final sourdough-based products. Sourdough biotechnology is an opportunity to promote the public health and the sustainable manufacture of local, healthy, natural, low-processed baking goods. These high-added value baking goods may improve population's diet and bring opportunities and competitiveness to the industry and local economy. To that purpose, it is fundamental to get a thorough knowledge of the adventitious microbiota present in the flours and sourdough, as well as the dynamics of sourdough fermentations. Indeed, the knowledge on microbial dynamics of sourdough starter cultures in such natural and complex matrixes as are sourdoughs is still embryonic. Therefore, dynamics of single and co-culture fermentations with microorganisms isolated from sourdoughs need to be better evaluated and modelled, and the environmental and growth conditions optimized to improve technological, nutritional and health attributes of sourdoughs. Moreover, the specific metabolic pathways and interactions between microorganisms, and driving forces and mechanisms that keep sourdough-based microbial consortia stable and productive needs to be better elucidated by interactomics. Increase the number of starter cultures (single or co-cultured) in the market is also a need, so that a greater diversity and quality of products in the global market may be achieved, and the consumption of sourdough baking products may be expanded and generalized.

In the last 45 years (since late 1970's), sourdough biotechnology has been intensively studied in different countries but deepest scientific knowledge on the technological and functional impact of sourdough fermentation is still open. Research on sourdough technology is definitively a hotspot in biotechnology in Europe and worldwide, which is perceptible by the scientific activity and production and the number of research groups focused on this field. Such a valuable scientific knowledge is dispersed and the recent COST Action 18101 (2018-2023) (COST Action 18101, 2018) entitled SOURDOMICS – *Sourdough biotechnology network towards novel, healthier and sustainable food and bioprocesses*, with hundreds of reseachers and several companies from all the Continents, is a good example of the importance of combining scientific and technological efforts between research groups and, also, highlights the importance given to the field of sourdough biotechnology. These kind of networks are a driving force to aggregate and valorize the dispersed scientific and technological knowledge on sourdough biotechnology, to share knowledge and laboratorial facilities, to promove multidisciplinary within research groups, to avoid duplication of research efforts, and to accelerate innovation and technological transfer.

Particularly, the studies on sourdough biotechnology have been mainly focused on the predominant yeasts and lactic acid bacteria but other microorganisms exist in the spontaneous sourdough fermentation and their role and impact on breadmaking is still not well-known. Examples are the ubiquitous *Bacillus* and the Gram-positive catalase-negative cocci lactic acid bacteria present in sourdoughs. There is still room to continue the research work on sourdough biotechnology involving a comprehensive genotype characterization, by metagenomics, and phenotype characterization of the whole microbiota present in cereals and sourdoughs, as well as involving the knowledge of the encrypted genetic potential and environmental expression of genetic traits and subsequent selection of microorganisms according to their health, nutritional and technological attributes. As a matter of fact, these microorganisms may be seen as cell factories to produce microbial functional (biological activity or technological functionality) metabolites and the metabolic pathways involved in the conversion of different substrates into high-added value functional metabolites need to be elucidated via metabolomics.

A deepest scientifically-sound characterization of sourdoughs and sourdough breads is essential to improve their quality, to better understand, control and optimize the sourdough fermentation and baking processes, and to support the health claims related to its consumption. Such knowledge will, consequently, help to expand market niches, to contribute to the economic added-value, and to trigger the integration of small farmers and households into the agri-food chain, as well as to enable the agricultural activity throughout the whole territories. Such scientific knowledge will contribute to the expansion of this market niche and, therefore, will contribute to the increase of its economic value, to the baking companies better compete in a globalizing market, to influence consumers' preferences and market orientations, to the consumer's protection and public health, and to the preservation of the authenticity of traditional food products.

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