

## CHAPTER 11

# Sourdough and cereal-based foods: Traditional and innovative products

Luca Settanni

*Department of Agricultural and Forest Sciences, University of Palermo, Italy*

Cereal-based foods have been key components of human diets for thousands of years (Alfonzo *et al.* 2013); several historical sources evidence that baking of leavened doughs was a daily practice in several cultures (Corsetti and Settanni 2007). Furthermore, as revealed by the discovery of fossil kernels, humans' utilization of cereals commenced in the Neolithic era (Settanni and Moschetti 2010). Cereals remain a major source of nutrition, particularly in developing and overpopulated countries (Blandino *et al.* 2003). Indeed, the history of several cultures is directly defined by cereals and, consequently, many human populations are identified by the cereals they eat: Chinese are 'rice people', South and Central Americans are 'maize people', North Americans and Mediterranean people are 'wheat people', North Europeans are 'oats and rye people' and Africans are 'millet and sorghum people' (Gifford and Baer-Sinnot 2007).

Millennia BCE, wheat was already one of the most important crops grown in the Mediterranean basin. Following mass migrations, its cultivation underwent a huge expansion, resulting in its production worldwide (Toderi 1989). Among cereals, wheat is critical in the Mediterranean diet: it provides approximately one-third of the daily protein and energy requirements (~2400 kcal) for an adult (Cannella and Piredda 2006). For this reason, wheat surpasses other cereals in terms of the number of hectares dedicated to its cultivation worldwide (Gifford and Baer-Sinnot 2007). However, the world's major sources of energy for humans are rice, wheat and maize (Spiertz and Ewert 2009).

Due to their potential for nutritional enhancement and the fact that their consumption substantially lowers the risk of significant diet-related diseases (Topping 2007), cereals also assume a basic role in the diet of industrialized countries. They are generally consumed after boiling or after fermentation.



In the latter case, cereals are first ground and mixed with water (Salovaara 1998). In this matrix, different microbial groups, including mainly bacteria and yeasts, transform raw materials into final products (Galati *et al.* 2014).

As already mentioned, the process of cereal fermentation has been important for humans since ancient times (Spicher 1999; De Vuyst and Neysens 2005). The acidic product, depending on the proportion of water, may represent a food or a beverage; sometimes it undergoes baking. Either way, fermentation contributes to the microbial stability of the final products (Mensah 1997). Hence, fermented cereal-based foods are complex microbial ecosystems, whose activities confer on the resulting products characteristic features such as palatability, high sensory quality, structure and texture, stability, nutritional and healthful qualities (Corsetti and Settanni 2007) and, when they are in a living form at the moment of consumption (unbaked products), potential probiotic properties (Perricone *et al.* 2014).

Traditional fermented foods prepared from most common types of cereals (such as rice, wheat, maize or sorghum) are well known in many parts of the world. In general, most of these foods are typical to restricted geographical areas, where the cereal substrates (alone or mixed with other cereals or legumes or tubers), in the form of flour, are processed into different products (Blandino *et al.* 2003). Among these foods, bread is common to many societies; it is produced almost everywhere, even in southeast Asian countries that have not been traditional bread consumers (Jenson 1998).

Products derived from cereal flours (e.g. bread, cereal snacks and breakfast cereals) are useful food vehicles to provide micronutrients, but sometimes the amount needed for correct alimentation is quite limited. For this reason, policy and programme responses of several countries, including those located in developing areas, promote food-based strategies, such as food fortification, to prevent micronutrient malnutrition. Food fortification allows delivery of the required nutrients to many populations without requiring radical changes in food consumption (Allen *et al.* 2006).

Bread is mainly produced from wheat, but rye and barley are often used for this purpose. Due to their gluten content, these cereals are toxic to people affected by coeliac sprue (CS), also known as gluten-sensitive enteropathy, an autoimmune disease of the small intestinal mucosa (Silano and De Vincenzi 1999). Although several attempts are being made to decrease the CS-inducing effects of gluten by enzymatic treatment (Caputo *et al.* 2010), to date a strict, life-long gluten-free diet is the only safe and efficient treatment available for this disease (Tack *et al.* 2010). For this reason, the development or enhancement of gluten-free products continues to grow (O'Shea *et al.* 2014). Due to the lower-quality characteristics of these products compared to those made with wheat flour, several studies are in progress to determine the best flour combinations and, for fermented foods, to select the starter strains able to enhance the quality



aspects of gluten-free products. Another strategy to produce products compatible with a CS diet is to hydrolyse the toxic components of flours that contain gluten. From this standpoint, many attempts have been made to apply different microorganisms, mainly lactic acid bacteria (LAB) and microbial proteases (Rizzello *et al.* 2014b), but much more work still needs to be done before the efficacy and effectiveness of the microbial activities are proven to obtain safe foods for CS-affected people.

### Cereals used in fermented food production

To date, more than half of the arable land in the world has been planted with cereals, mainly wheat, rice, maize, barley, sorghum and millet (Toderi 1993). Wheat clearly dominates in terms of hectares dedicated to its cultivation. However, thanks to improved plant breeding, rice production per hectare is higher than that for wheat. Other cereal crops are also relevant in several areas throughout the world (Dahlberg 2007).

Thanks to the great adaptability of its several varieties to different climatic conditions, including extreme values of temperature and/or humidity, wheat is cultivated almost everywhere for food and feed production. The species most commonly involved in food production are *Triticum durum* and *Triticum aestivum* (Toderi 1993). *T. durum*, which requires high temperatures, is cultivated in temperate regions, while *T. aestivum*, well suited to lower temperatures, is mainly cultivated in colder areas. In Italy, where cereal-based foods constitute the major part of the daily diet, *T. durum* and *T. aestivum* are traditionally used for pasta and bakery products (bread and leavened baked goods, such as breakfast or recurrent products), respectively. However, in some southern regions, durum wheat flour is used alone or in combination with soft wheat flour for bread production (Settanni *et al.* 2008).

The reason for the great expansion of wheat cultivation is that the other cereals are not as suitable for the production of foods characterized by nutritional value, shelf life and taste as those obtained from wheat flour (Macrae *et al.* 1993; Francis 2000; Cannella *et al.* 2010). In addition to wheat, rye is particularly common for bread production in Scandinavian countries, Germany, Poland and Russia (Bushuk 2001), maize is used in Portugal (Rocha and Malcata 1999) and sorghum in Sudan (Hamad *et al.* 1997), while rice is commonly used for gluten-free bread production (Neumann and Bruemmer 1997; Meroth *et al.* 2004).

The most cultivated cereal in developing countries is maize (Giardini and Vecchiellini 2000). It has been used for human consumption for centuries. The different varieties belonging to the species *Zea mays* are mainly distinguished by colour, basically white or yellow. The latter cultivars are particularly rich in carotenoids (pro-vitamin A; Schober and Bean 2008). The current maize



production could be considered sufficient to meet the caloric needs of nearly 2 billion people (Giardini and Vecchiellini 2000). The peculiar element of maize is starch, which is used in the confectionery industry.

In Asia and Africa, sorghum constitutes an integral part of the basic diet for millions of people (Schober and Bean 2008). Millet is considered to be the oldest cereal, widely cultivated in Asia and Africa and in some areas of eastern Europe (Taylor and Emmambux 2008). Barley is the main food source for a large number of people living in cold or semiarid areas where wheat does not fit (Mosca and Toniolo 2000).

The cereals mentioned are used to produce fermented products throughout the world (Blandino *et al.* 2003; De Vuyst and Neysens 2005; Galati *et al.* 2014). In particular, millet, maize, sorghum, rice and wheat are mainly used in Africa; maize, rice and wheat in America and Australia; wheat, maize, rye, barley and millet in Europe; rice, millet and wheat in Asia.

### Technological properties of cereal flours

The choice of raw materials is crucial to obtain bakery products able to satisfy "consumers' needs", and this is particularly important in breadmaking. For this purpose, wheat cultivars have been selected by bread producers since ancient times (Bottega *et al.* 2010). The technological aptitudes of wheat flour depend on its ability to form gluten, defining of its versatility. Gluten is generated by the interaction between two groups of proteins, gliadins and glutenins, when flour is mixed with water and the mixture is allowed to stand for a while (Cannella *et al.* 2010). The resulting dough is characterized by a viscoelastic behaviour that is responsible for its extensibility during kneading, leavening and the early stages of cooking, but also confers toughness and elasticity, which allow it to maintain its shape and to develop mass with regularity (Bottega *et al.* 2010). Although all wheat varieties are able to form gluten, the presence of particular protein subunits (different in structure and relationship gliadin/glutenin, composition and molecular weight) ensures the formation of tenacious nets (MacRitchie 1992; Shewry 2003; Bottega *et al.* 2010).

Other cereals, such as rye, contain protein subunits similar to those of wheat, but their ability to expand to give a well-developed leavened product is limited (He and Hosney 1991; Flander *et al.* 2007). Even more critical is the workability of wheat dough enriched with gluten-free flours (Mariotti *et al.* 2006, 2008; Schoenlechner *et al.* 2006). These flours negatively influence the leavening of the dough. Doughs made from durum wheat semolina are characterized by high strength and limited extensibility (Pogna *et al.* 1996). During leavening, therefore, dough tends to have a development by volume lower than that of a good soft wheat flour (Raffo *et al.* 2003).



## Cereal microflora

Cereal grains are naturally contaminated by eukaryotic (moulds and yeasts) and procaryotic (bacteria) organisms. The total microbial population and the relative species proportion on wheat grains can be affected by many factors, mainly climatic conditions such as temperature and rainfall, physical damage due to insects or mould attacks and application of insecticides and fungicides. Microorganisms of grains might follow different phases of flour preparation and, since flour does not undergo thermal treatment, it is a source of living and active microorganisms that can be found in the resulting fermented foods (Corsetti and Settanni 2007). The microorganisms that contaminate cereals are generally concentrated in the outer layers of kernels, and they tend to stay in fractions rich in bran during milling. Consequently, flour obtained from milling should theoretically contain a lower bacterial load than caryopses, but the subsequent conditioning phase can increase its microbial content (Berghofer *et al.* 2003).

The levels of living microorganisms present on cereals might range between  $10^4$  and  $10^7$  colony forming units (cfu) per gram, while they reach cell densities up to  $10^6$  cfu/g in the corresponding flours (Stolz 1999). The bacteria, mainly mesophilic, include Gram-negative aerobes (e.g. *Pseudomonas*) and facultative anaerobes (*Enterobacteriaceae*) and Gram-positive species (De Vuyst and Neysens 2005; Minervini *et al.* 2014). Among the latter bacterial groups, LAB, which together with yeasts are relevant for the process of food fermentation, might be found in several spontaneously fermented cereal-based products (Galati *et al.* 2014).

Although several studies have focused on the identification and characterization of LAB in the final fermented products, only a few works have investigated the microbial ecology of raw materials used for cereal-based food production. Some studies are available on the cereals, and the corresponding flours, used in breadmaking. The first document dates to 1987 (Galli and Franzetti 1987), when several samples of Italian wheat flours were analysed for the presence of different microbial groups. Subsequently, Corsetti *et al.* (1996) isolated and identified LAB and yeasts from common wheat and organic flours. Both studies were performed with a phenotypical/biochemical approach that revealed several lactobacilli (*Lactobacillus alimentarius*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus confusus* and *Lactobacillus viridescens*), even though some species were then reclassified as weissellas (*Weissella confusa* and *Weissella viridescens*).

The cultivable LAB populations associated with durum wheat kernels, cultivated in several Italian regions, as well as bran and non-conventional flours (amaranth, chickpea, maize, rice, quinoa and potato) used to produce gluten-free baked goods, were found at levels ranging between 1.00 and 2.16 log cfu/g. The isolates were genetically investigated by applying a polyphasic



strategy consisting of randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis, partial 16S rRNA gene sequencing, species-specific and multiplex PCRs (Corsetti *et al.* 2007a). Besides *Lactobacillus* (*Lactobacillus coryniformis*, *Lactobacillus curvatus* and *Lactobacillus graminis*), the work revealed the presence of other LAB genera: *Aerococcus* (*Aerococcus viridans*), *Lactococcus* (*Lactococcus garvieae*), *Enterococcus* (*Enterococcus durans*, *Enterococcus casseliflavus*, *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus mundtii*) and *Pediococcus* (*Pediococcus pentosaceus*). De Vuyst and Neysens (2005) reviewed the presence of several LAB species (*Lactobacillus casei*, *Lb. coryniformis*, *Lb. curvatus*, *Lb. plantarum*, *Lactobacillus salivarius*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Ent. faecalis*, *Lactococcus lactis*, *Pediococcus acidilactici*, *Pediococcus parvulus*, *Pe. pentosaceus*, *Leuconostoc* and *Weissella*) on cereal grains.

More recently, Alfonzo *et al.* (2013) investigated different wheat (*T. durum* and *T. aestivum*) flours used for the production of traditional sourdough breads in Sicily (southern Italy) using culture-dependent phenotypical and genotypical tools, as well as a culture-independent method based on the denaturing gradient gel electrophoresis (DGGE) technique. The last approach was useful to test the technological performance of the dominant strains (which reached concentrations up to 4.75 log cfu/g) and also to detect the species present at undetectable (subdominant) levels and/or as dormant (non-cultivable) flora. *Ent. mundtii*, *Lactobacillus sanfranciscensis*, *Lb. plantarum*, *Lactobacillus sakei*, *Lc. lactis*, *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides*, *Leuconostoc citreum*, *Pe. pentosaceus*, *Weissella cibaria* and *We. confusa* were identified. In particular, the most prevalent species detected were *We. cibaria*, *Lb. plantarum*, *Ln. pseudomesenteroides* and *Ln. citreum*. DGGE analysis confirmed the detection of the genera to which most isolates belonged (*Lactobacillus*, *Enterococcus*, *Leuconostoc* and *Weissella*), but only two species, *Lb. plantarum* and *Ln. citreum*, were clearly identified. The apparent lack of correspondence between culture-dependent and culture-independent methods was explained by the fact that when bacteria isolated by plating are not detected with DGGE analysis based on 16S rRNA gene amplification, they are not a major component of the microbial community being investigated (Shinohara *et al.* 2011). For this reason, a combined approach consisting of both methodologies provides the best strategy for detection of microbial communities within complex food matrices (Carraro *et al.* 2011).

Regarding eukaryotic organisms, yeasts are also detected both on the cereal surface and in flour samples ranging from a few cells to 10<sup>4</sup> and 10<sup>3</sup> cfu/g, respectively. The species most commonly found are *Candida*, *Cryptococcus*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Sporobolomyces*, *Torulasporea* and *Trichosporon*. Among fungi (circa 10<sup>4</sup> cfu/g), *Alternaria*, *Cladosporium*, *Drechslera*, *Fusarium*, *Helminthosporium* and *Ulocladium* (from the field), and *Aspergillus* and *Penicillium* (from storage), are found (De Vuyst and Neysens 2005).



Furthermore, it is also important to consider that the microorganisms performing the fermentation of cereal flours may originate from the equipment used in the milling and/or production process (Berghofer *et al.* 2003).

### Cereal fermentation

Since the beginning of human civilization there has been an intimate relationship between the human being and the fermentative activities of microorganisms. These activities have been utilized in the production of fermented foods and beverages, which are defined as those products that have been subordinated to the effect of microorganisms or enzymes determining desirable biochemical changes (Settanni and Moschetti 2014). Fermentation represents the oldest and most economical method of producing and preserving food (Chavan and Kadam 1989; Billings 1998). In fact, fermentation helps in the production of safe and stable foods with a longer shelf life than their raw materials. These foods are more digestible and appealing than unprocessed substrates because they acquire new desired organoleptic characteristics (Settanni and Moschetti 2014). In addition, fermentation provides a natural way to reduce the volume of the material to be transported, to destroy undesirable components, to enhance the nutritive value and to reduce the energy required for cooking (Simango 1997). During cereal fermentation several volatile compounds are formed, which contribute to a complex blend of flavours in the processed products (Chavan and Kadam 1989).

The microorganisms responsible for fermentation may be the microflora indigenously present on the substrate (and this occurred unknowingly for millennia) or they may be added as starter cultures (Harlander 1992). The latter strategy commenced when the microorganisms had been isolated and their activities discovered and studied.

By one biochemical definition, fermentation is an anaerobic process for deriving energy from the oxidation of organic compounds using an endogenous electron acceptor, which is usually an organic compound (Prescott *et al.* 2005). Following this process, the carbohydrates are partially oxidized and several microorganisms produce energy by means of this metabolic pathway. Among the several fermentations employed to produce different foods (Soni and Sandhu 1990), the two main processes that are defining for the transformation of cereal flours are alcoholic and lactic acid fermentations. Alcohol fermentation results in the production of ethanol and yeasts are the predominant organisms involved; lactic acid fermentation is carried out by LAB (Corsetti and Settanni 2007).

In general, natural fermentation of cereals leads to a decrease in the level of carbohydrates as well as some non-digestible poly- and oligosaccharides. This process determines the saccharification of starch and increases the availability of



proteins (Blandino *et al.* 2003). Certain amino acids may be synthesized and the availability of B-group vitamins might be improved. Fermentation also provides optimum pH conditions for the enzymatic degradation of phytate, which is present in cereals in the form of complexes with polyvalent cations such as iron, zinc, calcium, magnesium and proteins. Such a reduction in phytate may increase the amount of soluble iron, zinc and calcium severalfold (Haard *et al.* 1999; De Angelis *et al.* 2003).

## Cereal-based fermented products

Based on the type of flour (alone or in combination), the fermenting agent(s) and the technological process(es) applied, different products can be obtained. The fermenting microorganisms for almost all cereal-based fermented products are mainly LAB and a few yeast species. In several cases, their combined action allows the production of foods and beverages with the desired quality characteristics (Galati *et al.* 2014). However, the microbial populations responsible for the fermentation of several niche cereal-based products, especially those produced in countries where this process is not driven by selected starter cultures, remain unknown.

Cereal-based fermented foods are spread all over the world and undoubtedly bread is the main product. Bread is the typical and oldest food of leavened products and it is a symbol of religion. The earliest records date back to the second millennium BCE, when, after a flood of the Nile that covered the grain reserves, the Egyptians realized that grain flour when mixed with water increased in volume over time (Di Giandomenico 2010). During the Second World War, due to the scarcity of cereals, a large number of cereals (maize, rye, oat, barley, rice, sorghum) or other vegetable sources (bean, cassava, soy, potato, chestnut flour etc.) have been used in breadmaking in place of wheat (INSOR 2012).

To date, unlike bread, where biotechnologies are under control during the transformation process carried out at an industrial level, the preparation of many other cereal-based fermented foods is restricted to limited areas and they are mostly produced on a domestic scale (Owczarek *et al.* 2004). However, some of them are particularly important in different countries and have been the object of study for many research groups (Table 11.1).

### Bread

The term 'bread' refers to a food of any shape and dimension obtained from a dough prepared with flour and water, with or without salt, fermented naturally or with the addition of yeasts and subsequently subjected to cooking. Breadmaking technology (Figure 11.1) is quite simple (Pagani *et al.* 2006).

The production of leavened bakery products can be summarized as the semi-solid mass transformation of the dough, a particular 'emulsion', characterized by



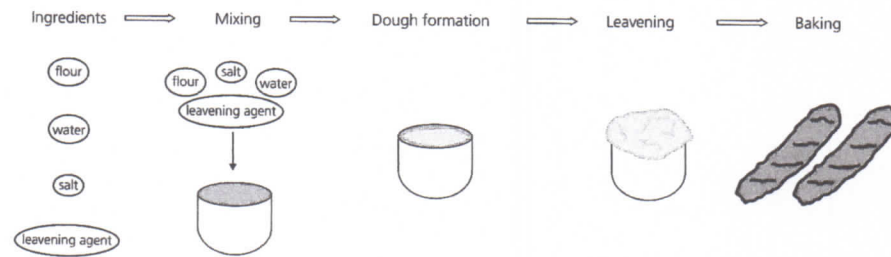
**Table 11.1** Traditional fermented cereal-based foods and beverages that have been the object of scientific investigation.

Products	Cereals	Countries
Amgba	Sorghum, millet	Cameroon, Chad
Ang-kak	Rice	China, Indonesia, Thailand
Atole	Maize	Guatemala, Mexico
Ben saalga	Millet	Burkina Faso
Boza	Wheat, millet, maize	Albania, Bulgaria, Romania, Turkey
Bread	Wheat, rye, barley, sorghum, rice	Five continents
Bushera	Sorghum, millet	Uganda
Chicha	Maize	Peru
Chikokivana	Maize, millet	Zimbabwe
Chongju	Rice	Korea
Dolo	Sorghum	Burkina Faso
Ikikage	Sorghum	Rwanda
Jaanr	Millet	Northeastern Himalayas
Kachasu	Maize	Zimbabwe
Kaffir beer	Maize	South Africa
Koko	Maize	Ghana
Kunu-Zaki	Millet	Nigeria
Lao-chao	Rice	China, Indonesia
Mantou	Wheat	China
Mirin	Rice	Japan
Muramba	Sorghum	Uganda
Mutwiwa	Maize	Zimbabwe
Ogi	Maize, sorghum, millet	Nigeria, West Africa
Pito	Maize, sorghum	Ghana, Nigeria
Pozol	Maize	Mexico
Puto	Rice	Philippines
Sake	Rice	Japan
Sourdough bread	Wheat, rye, barley, sorghum, rice	Five continents
Takju	Rice, wheat	Korea
Tape ketan	Rice	Indonesia
Tapuy	Rice	Philippines
Tchoukoutou	Sorghum, millet, maize	Benin, Togo
Tesguino	Maize	Mexico
Togwa	Maize, sorghum, millet	Tanzania

a continuous phase represented by hydrated gluten that surrounds the starch granules in which are dispersed microbubbles of air, in a 'foam', that is to say in a product in which the continuous phase retains significant volumes of gas (Bottega *et al.* 2010).

The leavening process is of paramount importance during breadmaking. It determines the increase of dough volume, the development of precursors of the aroma compounds and the improvement of the nutritional characteristics of flour.





**Figure 11.1** General process of bread production.

The volume expansion can be achieved in different ways: the biological approach (thanks to carbon dioxide [CO<sub>2</sub>] resulting from yeasts and/or LAB metabolism); the use of chemicals added to the formulation (which exert their action especially during cooking); and the physical approach, with the inclusion of air as a result of intensive mechanical action and typical leavening of some formulations rich in fat (Bottega *et al.* 2010).

Biological leavening assumes a basic importance for the sensory properties of the final product. This process can be carried out by baker's yeast, mainly represented by *Saccharomyces cerevisiae*, and a mixture of yeasts and LAB. In the latter case, the technology applied is that of sourdough, referred to as a mixture of flour and water in which the development of LAB results in the production of lactic acid and acetic acid. LAB developing in the dough may originate as contaminants of flour and/or the bakery environment or might derive from a starter culture containing one or more known species of LAB (De Vuyst and Neysens 2005).

### Sourdough

Sourdough is a mixture of cereal flour and water in which a heterogeneous population composed of LAB and yeasts is metabolically active, either by spontaneous fermentation or by fermentation initiated through the addition of a sourdough starter culture, whether or not it involves backslopping (De Vuyst *et al.* 2009).

Based on the technology applied, sourdoughs have been grouped into type I, type II and type III (Böcker *et al.* 1995). Type I sourdoughs are traditionally characterized by continuous, daily refreshment to keep the microorganisms in an active state; fermentation is carried out at room temperature until the pH reaches a final value of around 4.0 (Corsetti and Settanni 2007). Type II sourdoughs are semifluid silo preparations fermented at temperatures higher than 30 °C for long periods (at least 2 days); these sourdoughs are generally added during bread preparation as dough-souring supplements (Böcker *et al.* 1995; Hammes and Gänzle 1998). Type III sourdoughs are dried preparations containing LAB resistant to the drying process (Hammes and Gänzle 1998). Types II and III sourdoughs require the addition of *S. cerevisiae* as a leavening agent. Some authors



(De Vuyst and Neysens 2005) also reported on type 0 dough. This product, although prepared exclusively from baker's yeast and not made with sourdough technology, also contains LAB species as contaminants of the yeast inoculums. These are especially lactobacilli rather than *Pediococcus*, *Lactococcus* and *Leuconostoc* spp. (Jenson 1998), and contribute only to a small degree to the acidification and aroma development of dough because of the short processing time.

The addition of sourdough improves the texture, flavour, nutritional aspects and shelf life (Gänzle *et al.* 2007) of wheat bread produced from baker's yeast due to the synthesis of aroma compounds (Czerny and Schieberle 2002; Hansen and Schieberle 2005), enzymes and antibacterial (Settanni *et al.* 2005) and anti-fungal (Ryan *et al.* 2008; Poutanen *et al.* 2009) compounds during fermentation. Sourdough fermentation has been demonstrated also to enhance the sensory quality of rye breads (Rizzello *et al.* 2014b).

Besides the technological and nutritional aspects, sourdough fermentation may also provide several health benefits, such as a decrease of the glycemic response of baked goods, enhancement of the content of bioactive compounds and increase of mineral uptake (Gobbetti *et al.* 2013). Compared with sourdough bread, white wheat bread started with baker's yeast alone shows some drawbacks such as low protein digestibility, high carbohydrate content, high glycemic index, low-resistant starch and low level of dietary fibre (Dhinda *et al.* 2011).

### Sourdough microorganisms

The proportions of LAB and yeasts in sourdough are not random, but rather respect a defined relationship. The ratio between LAB and yeasts is generally reported to be 100:1 (Gobbetti *et al.* 1994; Ottogalli *et al.* 1996). This could be the result of direct interaction between these microbial groups.

The study of the social activities exhibited by microorganisms is defined as 'sociomicrobiology' (Parsek and Greenberg 2005). The microorganisms were for a long time believed to exist as single cells in a given environment searching for nutrients to multiply. Bacteria were the first microbes to be studied in order to decipher their code of communication. It has been discovered that they are active in performing a census of their population, as well as in investigating the environment for development and in feeling the presence of competitors (Fuqua *et al.* 1996). Such actions are the result of efficient intercellular communication that is based on the production, release and detection of and reply to small signal molecules, which accumulate and trigger cascade events when a 'quorum' concentration is reached. Hence, the term 'quorum sensing' is used to describe cell-cell communication. Based on this system the bacteria can 'count' one another (Fuqua *et al.* 1994), acting as a group. Recently, it became apparent that fungi, like bacteria, also use quorum regulation to affect population-level behaviours. Furthermore, considering the extent to which quorum-sensing regulation



controls important processes in many distantly related bacterial genera, it is not surprising that cell density-dependent regulation also appears to be prevalent in diverse fungal species (Hogan 2006).

The studies regarding cell-cell communication in sourdough ecosystems are so far limited to the group of LAB (Di Cagno *et al.* 2007, 2010), but this topic is under study to elucidate the mechanisms of interdomain communication between LAB and yeasts.

### Yeasts

Several sourdoughs are reported to host *S. cerevisiae*. This finding is generally due to introduction through the addition of baker's yeast (Corsetti *et al.* 2001) or to cross-contamination in bakeries where both conventional and sourdough breads are produced (Valmorri *et al.* 2010). Yeasts found in sourdoughs belong to different genera (Rossi 1996; Stolz 1999; Gullo *et al.* 2002). The typical sourdough yeasts are *Saccharomyces exiguus*, *Candida humilis* (formerly described as *Candida milleri*) and *Issatchenkia orientalis* (*Candida krusei*; Garofalo *et al.* 2008; Iacumin *et al.* 2009). Other yeast species detected in sourdough ecosystems are *Pichia anomala* (as *Hansenula anomala*), *Saturnispora saitoi* (as *Pichia saitoi*), *Torulaspora delbrueckii*, *Debaryomyces hansenii*, *Pichia membranifaciens* (Corsetti and Settanni 2007) and *Candida famata* (Mohamed *et al.* 2007). The extensive variability in the number and type of species found depends on several factors, dough yield (weight of dough/weight of flour  $\times$  100), type of cereal used, temperature of fermentation and temperature for sourdough maintenance (Gobbetti *et al.* 1994). Yeasts via alcoholic fermentation are primarily responsible for the leavening of dough (Corsetti and Settanni 2007).

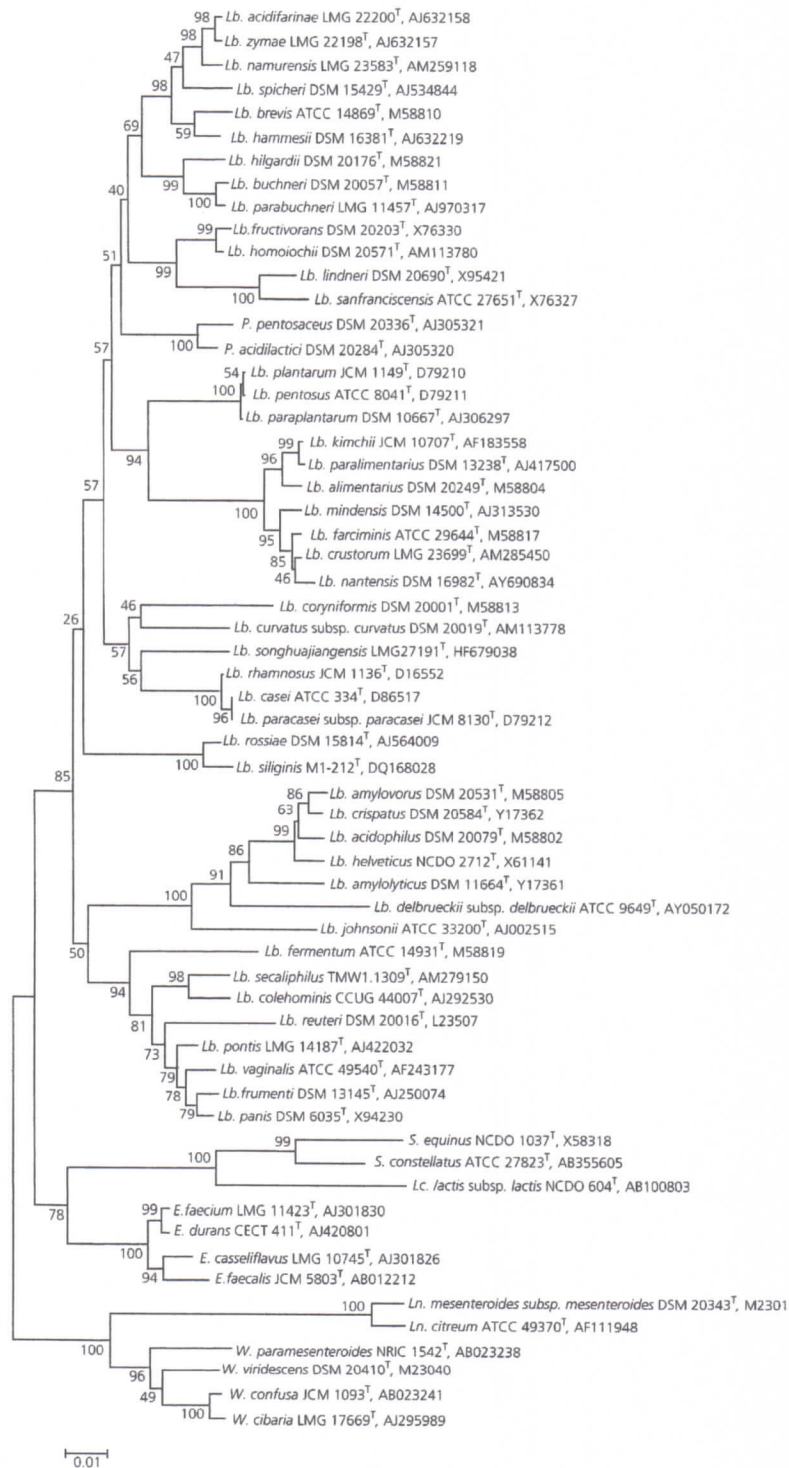
### Lactic acid bacteria

LAB are involved in the process of acidification of sourdough, but heterofermentative species partly contribute to the mass blowing (Gobbetti *et al.* 1995).

Unlike other fermented foods, where LAB responsible for the transformation of raw materials into final products belong to obligate homofermentative and/or facultative heterofermentative species, obligate heterofermentative species play a major role in sourdough (Salovaara 1998), especially when sourdoughs are prepared in a traditional manner (Corsetti *et al.* 2001, 2003). Typical sourdough LAB mainly belong to the genus *Lactobacillus* and include all three metabolic groups discussed (Hammes and Vogel 1995). However, other LAB belonging to *Leuconostoc*, *Weissella*, *Pediococcus*, *Lactococcus*, *Enterococcus* and *Streptococcus* genera have been isolated from sourdough (Corsetti and Settanni 2007).

Most of the LAB species commonly detected in sourdough (Figure 11.2) have sourdough as their primary and sole source of isolation, probably because no other ecosystem can support their growth. For instance, except sourdough, no other habitat is known for *Lactobacillus sanfranciscensis* (Hammes *et al.* 2005).





**Figure 11.2** Phylogenetic tree of LAB commonly associated with or found in sourdough products based on 16S rRNA gene sequences. Sequence alignment was performed with CLUSTALX (Thompson *et al.* 1997). Sequence and alignment manipulations and calculation of similarity values and nucleotide compositions of sequences were performed with the GeneDoc program version 2.5.000 (K.B. Nicholas and H.B. Nicholas, unpublished data). Positions available for analysis were circa 1150 bp. Phylogenetic and molecular evolutionary analysis was conducted using MEGA version 3.1 (Kumar *et al.* 2004). Bar 0.01 nucleotide substitution per site.



Some of the species associated with this environment have been misidentified because of the lack of application of molecular methods, which were unavailable in the past, or have been found in sourdough due to cross-contamination.

Some non-*Lactobacillus* species isolated from sourdough have only been detected at subdominant levels (Corsetti *et al.* 2007b). As an example, in mature Italian type I sourdough, *E. faecium* and *P. pentosaceus* have been found in the range  $10^4$ – $10^6$  cfu/g, while lactobacilli were about 2–3 orders of magnitude higher (Valmorri *et al.* 2006). Corsetti *et al.* (2007b) investigated the role of *Ent. faecium* and *Pe. pentosaceus* during sourdough preparation. Strains of both species were followed in dual combination with *Lb. sanfranciscensis*. During the first steps of sourdough preparation, single inocula of *Ent. faecium* and *Pe. pentosaceus* determined a stronger and more rapid acidification of dough than the *Lb. sanfranciscensis* strain used. Subsequently, the behaviour monitored during the co-fermentation of *Ent. faecium/Lb. sanfranciscensis* and *Pe. pentosaceus/Lb. sanfranciscensis* showed that *Ent. faecium* and *Pe. pentosaceus* prepare the environment for the establishment of the typical species of mature sourdough, including *Lb. sanfranciscensis*, by lowering the pH.

Minervini *et al.* (2014) described the typical LAB population dynamic in sourdough, referred to as 'three-phase evolution', regardless of the type of flour. The three phases indicated are the dominance of LAB species belonging to the genera *Enterococcus*, *Lactococcus* and *Leuconostoc*; the increasingly important presence of sourdough-specific LAB, such as species belonging to the genera *Lactobacillus*, *Pediococcus* and *Weissella*; and the dominance of well-adapted sourdough strains, belonging to obligate heterofermentative species such as *Lb. sanfranciscensis*, *Lb. fermentum* and *Lactobacillus pontis* and to the facultative heterofermentative *Lb. plantarum* (Gänzle *et al.* 2007), although the presence of some *Leuconostoc* spp. is sometimes revealed. This succession of LAB is mainly driven by different tolerance to acidic conditions and to different adaptation mechanisms related to carbohydrate and nitrogen metabolism (Gänzle *et al.* 2007). The establishment of obligate heterofermentative lactobacilli is essential for the optimal fermentation of traditional sourdough (Salovaara 1998).

Regarding cell–cell communication among lactobacilli in sourdough, Di Cagno *et al.* (2007) followed, by a proteomic approach, the growth of *Lb. sanfranciscensis* in mono-culture and co-culture with *Lb. plantarum*, *Lb. brevis* or *Lactobacillus rossiae*. When co-cultured, the *Lb. sanfranciscensis* strain, depending on the combination, overexpressed several proteins during the late stationary phase. The induced polypeptides, only in part common to all co-cultures, were identified as stress proteins, energy metabolism-related enzymes and proline dehydrogenase, GTP-binding protein, *S*-adenosyl-methyltransferase and Hpr phosphocarrier protein. Furthermore, two quorum-sensing genes involved, *luxS* and *metF*, were shown to be expressed in the *Lb. sanfranciscensis* strain studied. Later, the same research group studied the effect of pheromone plantaricin A produced by a *Lb. plantarum* strain (DC400) towards other sourdough LAB, and it was found that this

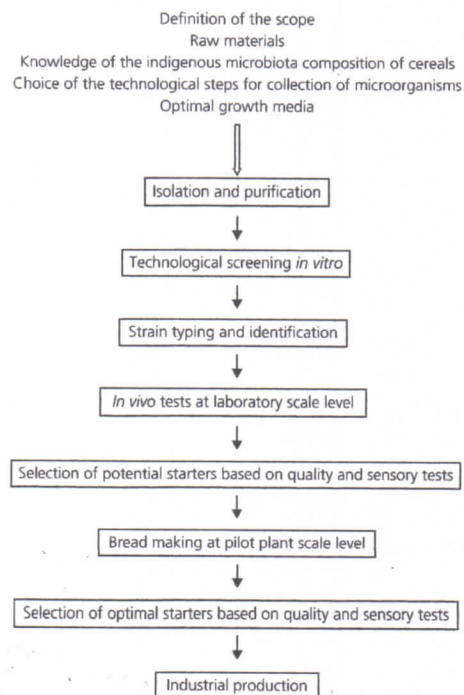


pheromone influenced the growth and survival of the other strains co-cultivated with *Lb. plantarum* DC400 differently (Di Cagno *et al.* 2010).

### Starter selection for sourdough production

In the last few years, several research groups have focused their attention on the selection of strains to be used as starter cultures for controlled sourdough fermentation. Analysis of microbial biodiversity and monitoring the dominant microorganisms during fermentation is a key step for the selection of strains able to drive the transformation processes. The starter cultures for fermentation are selected based on their specific technological traits in order to obtain final products with a set of desired characteristics (Figure 11.3).

Generally, LAB are primarily tested for their rapid acidification, whereas yeasts are first tested for their alcoholic fermentation rate. However, depending on the type of bread to be produced, LAB are commonly selected also for other technological performance, such as contribution to the development of the flavour and structure of the dough, reduction of antinutritional factors and, in order to determine microbial stability during fermentation and elongate the



**Figure 11.3** Schematic representation of the plan for selection of starter culture(s) to be used for sourdough fermentation.



shelf life of the final products, for their competitiveness with undesired microorganisms based on the production of secondary metabolites, mainly bacteriocins and antifungal compounds (Corsetti and Settanni 2007).

The selection of LAB is performed during fermentation; this allows the collection of dominant strains that have competitive advantages over the native microbiota. The dominant strains will ensure, to a certain extent, the successful transformation of flour into sourdough, thanks to their adaptation to the specific fermentation technology, the environmental conditions and the availability of substrates. Once the dominant isolates are collected, their performance is evaluated *in vitro*, first using the optimal commercial synthetic media and then using sterilized media prepared from raw material extracts that mimic real conditions, without interaction with indigenous microorganisms (Settanni *et al.* 2014). Subsequently, the isolates showing interesting properties are identified at strain and species levels. The different strains are tested *in vivo* using untreated flours, so that their performance is evaluated in the presence of the autochthonous microbiota, and their capacity to dominate the microbial community is monitored (Alfonzo *et al.* 2013). The final products are generally subjected to sensory and quality evaluations to select the best strains, in individual and/or multiple combinations, able to recreate the traditional aromatic profile, in order to obtain breads with the desired characteristics constant over time (Settanni *et al.* 2014).

### **Fortified fermented cereal-based products**

Food fortification refers to the addition of micronutrients to processed foods. It represents a valid technology for reducing micronutrient malnutrition when and where existing food supplies and limited access fail to provide adequate levels of the respective nutrients in the diet. In industrialized countries, food fortification has long been used for the successful control of deficiencies of vitamins A and D, several B vitamins (thiamine, riboflavin and niacin), iodine and iron. From the early 1940s onwards, the fortification of cereal products with thiamine, riboflavin and niacin became common practice (Allen *et al.* 2006). Fortified breads are obtained through the enrichment of flour with nutrients. In fact, the nutritional features of white wheat are quite limited. This is due to the low levels of essential amino acids, such as lysine, and dietary fibre in white flour (Dhinda *et al.* 2011). For years, the most common constituents added to these kind of breads have been folic acid and iron. However, in the last few years the addition of alternative plant-based protein sources has become usual. It is becoming common practice in white bread production to use dietary fibre and ingredients or by-products rich in fibre (De Angelis *et al.* 2007, 2009; Rizzello *et al.* 2012), like a mixture of soy proteins, oat bran and legume flours (Sadowska *et al.* 2003; Kamaljit *et al.* 2010; Dhinda *et al.* 2011; Mohammed *et al.* 2012; Rizzello *et al.* 2014a) to enhance its nutritional value.



These products are gaining importance not only because of the increasing number of people on a vegetarian diet, but also due to the high energy requirements for animal protein production, particularly relevant for people who have no access to an animal protein-rich diet. For example, besides bread, several products are produced in different areas, especially Africa and Asia, with cereals in combination with legumes (Blandino *et al.* 2003), thus improving the overall protein quality of the fermented products. As reported earlier, cereals are deficient in lysine, but are rich in cysteine and methionine. Legumes, on the other hand, are rich in lysine but deficient in sulfur-containing amino acids. Thus, by combining cereals with legumes, the overall protein quality is improved (Campbell-Platt 1994). The main non-bread products obtained from the fermentation of mixtures of cereals and other vegetables are listed in Table 11.2.

Several attempts are being made to fortify the common ingredients used in bread production. Since bread is mainly obtained via the fermentation of wheat flour sugars derived from starch involving chemical interactions of the various food components, these interactions can be adjusted to create desirable products only if the chemical and physical processes are well understood (Sivam *et al.* 2010).

The incorporation of legumes in novel, convenient and healthy food products (Schneider 2002; G6mez *et al.* 2008) represents a valuable strategy for increasing the global consumption of legumes, which is declining (Kohajdova *et al.* 2013) and is below the recommended amount (McCrory *et al.* 2010). For

**Table 11.2** Traditional foods and beverages obtained through fermentation of mixtures of cereals and other vegetable sources.

Products	Cereals and legumes	Countries
Adai	Cereals and legumes	India
Banku	Maize and cassava	Ghana
Chee-fan	Wheat and soybean	China
Dhokla	Rice, wheat and Bengal gram	India
Dosa	Rice and Bengal gram	India
Hamanatto	Wheat and soybean	Japan
Idli	Rice and black gram	India, Sri Lanka
Kanji	Rice and carrots	India
Kecap	Wheat and soybean	Indonesia
Miso	Rice and soybean	China, Japan
Munkoyo	Maize and roots of munkoyo	Africa
Shoyu	Wheat and soybean	China, Japan, Taiwan
Tao-si	Wheat and soybean	Philippines
Taotjo	Wheat, rice and soybean	India
Tarhana	Wheat and vegetables	Turkey
Tauco	Cereals and soybean	Indonesia
Vada	Cereals and legumes	India



this reason, some reports have proposed the addition of legumes to different products such as bread (Dhinda *et al.* 2011; Mohammed *et al.* 2012), biscuits (Eissa *et al.* 2007; Tiwari *et al.* 2011), cakes (Gómez *et al.* 2008), chapatti (Kadam *et al.* 2012) and crackers (Kohajdová *et al.* 2011).

When legume flours are used for breadmaking, the adjustment of several process parameters is needed to get a high sensory quality that is acceptable to the majority of consumers (Maninder *et al.* 2007; Kohajdová *et al.* 2013). One option to improve the sensory and functional quality of breads containing legume flours is represented by the use of sourdough fermentation. With this in mind, Rizzello *et al.* (2014a) developed a biotechnological protocol for manufacturing a white bread enriched with chickpea, lentil and bean flours through sourdough fermentation. For this purpose, the authors prepared type I sourdough containing legume flours according to traditional protocols routinely used for making typical Italian breads. LAB populations in wheat-legume sourdough included *Lb. plantarum*, *Lb. sanfranciscensis*, *Ln. mesenteroides*, *Lb. fermentum*, *We. cibaria*, *Lactobacillus pentosus*, *Lb. coryniformis*, *Lb. rossiae*, *Lb. brevis*, *Lactobacillus parabuchneri* and *Lactobacillus paraplantarum*, most of which are typically associated with mature sourdough. Compared with wheat-legume breads leavened with commercial yeasts, wheat-legume sourdough breads were characterized by higher quality parameters and acceptability by consumers, thus proving the defining role of sourdough technology in improving the characteristics of bakery products obtained with novel formulas.

## Gluten-free cereals

Gluten is toxic for people affected by CS. Gluten causes self-perpetuating mucosal inflammation and subsequent loss of absorptive villi and hyperplasia of the crypts. Proteolytic enzymes of the endoluminal tract acting on prolamins of wheat (a-, b-, c-, and x-gliadin), rye (secalin) and barley (hordein) produce proline- and glycine-rich polypeptides that are responsible for the disease (Silano and De Vincenzi 1999). Triticale also contains gluten (Wolter *et al.* 2014a). The list of proteins that liberate toxic peptides includes the high molecular weight glutenins (Dewar *et al.* 2006). Both gliadins and glutenins are rich in two amino acids, proline (very resistant to the hydrolysis process) and glutamine. The gluten, when ingested, goes through a process of digestion and the protein is not completely degraded by enzymes with prolyl endopeptidase activities (Catassi and Francavilla 2010). The gluten is degraded into small peptides and their further proteolysis is made difficult by the position and abundance of proline residues (Hausch *et al.* 2003). Some of these peptides have an immuno-genetic activity for subjects predisposed to develop CS (Catassi and Francavilla 2010). For these reasons, those affected by CS cannot ingest gluten-containing products.



Most of the food products present on the market that characterize the daily diet in many countries are made with gluten-containing cereals. The number of people affected by CS is high, thus the production of baked goods that can be consumed by these subjects is of paramount importance. The main raw materials used for the production of gluten-free leavened baked are rice (*Oryza sativa* and *Oryza glaberrima*), maize (*Zea mays*), sorghum (*Sorghum bicolor* L. Moench) and millet (*Panicum miliacum*) and pseudo-cereals such as amaranth (species of genus *Amaranthus*), quinoa (species of genus *Chenopodium*) and buckwheat (species of genus *Fagopyrum*; De Angelis and Di Cagno 2010). Rice is one of the most important cereals used for this purpose because the carbohydrate fraction of the flour consists predominantly of starch present in small granules. Amylopectin, easily digested, is present in higher amounts than amylose. When rice flour is used, it provides softness and stability. Maize finds several uses (e.g. polenta, cornflakes, tortillas, snacks etc.) in the diet of coeliac subjects, but it is scarcely used for the production of gluten-free bread. The use of sorghum flour is recommended in the diet of CS patients, as it is the species phylogenetically most distant from the other cereals. Millet flour does not show a good aptitude for baking and for leavened gluten-free production, therefore it is often used in combination with other flours (Schober *et al.* 2003). Quinoa and amaranth have been utilized for the manufacture of different bakery products (Lorenz and Coulter 1991; Nsimba *et al.* 2008) and quinoa flour is also added for the manufacture of enriched gluten-free bakery products (Taylor and Parker 2002).

Most of the gluten-free products present on the market are characterized by a lower quality than conventional products made from gluten-containing flours. Hence, the combination of gluten-free flours and the choice of their ratios is essential for the acceptability of the final products (De Angelis and Di Cagno 2010). Currently, the most utilized gluten-free flours include maize, potato and rice flour and starches, used as base flours due to their bland flavour and neutral effects on baked products. These flours and starches usually tend to be low in nutrition and have very minimal structure-building potential. Chestnut flour presents high potential in the development of gluten-free products (O'Shea *et al.* 2014).

### Gluten-free fermented products

The use of gluten-free flours represents a valid alternative to the complete absence of cereals in the diet of CS patients. However, the poor baking performance of these flours (due to the lack of gluten), the low nutritional quality and the poor sensory characteristics of the resulting products determine that there is an important technological challenge to be faced (Wolter *et al.* 2014a). Furthermore, gluten-free products have a limited microbial shelf life (Gallagher 2009; Hager *et al.* 2011).



In the case of bread, the use of sourdough as a bioprocessing ingredient in gluten-free formulations may provide several positive characteristics in the final product. O'Shea *et al.* (2014) summarized the beneficial role of sourdough in gluten-free bread production as follows:

- Production of peptidase able to detoxify the peptides responsible for CS when using wheat and rye flours.
- Activity against mycotoxins produced from fungi found on maize, sorghum and millet.
- Decrease of pH for degradation of phytic acid.
- Extraction of bioactive compounds from the flour and release of biomolecules that are part of the LAB/yeast metabolism.
- Elongation of shelf life.
- Enhancement of the flavour profile.
- Production of exopolysaccharides (EPS), proven to be prebiotic, that are useful to produce breads with a softer texture without the addition of hydrocolloid.

All the positive aspects of gluten-free breads observed with the use of sourdough are due to LAB communities, but their action is strain dependent (Arendt *et al.* 2011).

Among LAB, *Lb. plantarum* is reported to be dominant in several gluten-free sourdoughs produced from amaranth, buckwheat, quinoa, rice and teff flour (Vogelmann *et al.* 2009; Moroni *et al.* 2011a). Strains of this species have been proven to improve the staling rate and crumb hardness of brown rice, buckwheat-based, gluten-free formulations with the addition of sourdough and an inoculum size of  $10^8$  cfu/g (Moore *et al.* 2007). However, breads made from different gluten-free flours may exhibit an undesirable aroma (Hager *et al.* 2012), especially when compared to wheat sourdough breads, due to the absence or low intensity of a wheat bread-like note. In general, the characteristic odour attributes of gluten-free breads are pea-like with buckwheat, quinoa and teff flours, cooked potato-like with quinoa and teff flours, vomit-like with sorghum and teff flours and mouldy with buckwheat and quinoa flours (Wolter *et al.* 2014a). The odorants and the resulting undesirable notes cause a negative impact on the aroma quality of gluten-free breads.

Selected LAB strains are able to generate very specific volatile organic compounds in wheat sourdough (Settanni *et al.* 2013). Hence, the use of individual metabolic properties of LAB seems to be a promising approach to increase also the aroma quality of gluten-free breads, although the type of flour influences sourdough fermentation, affecting the availability of carbohydrates as primary fermentation substrates, nitrogen sources and growth factors such as vitamins, minerals and the buffering capacity (Hammes *et al.* 2005).

In order to include starter cultures in gluten-free sourdough, it is important to select the correct strain(s) for a given flour type (Rühmkorf *et al.* 2012a). To study the role of LAB, the effect of *Lb. plantarum* sourdough on gluten-free bread quality was evaluated using a composite recipe (Moore *et al.* 2008; Coda *et al.*



2010). This species has been used by Wolter *et al.* (2014a) to ferment single gluten-free (buckwheat, oat, quinoa, sorghum and teff) flours, in order to investigate the influence of the corresponding sourdoughs added for fermentation in the resulting breads. All sourdoughs decreased dough strength, resulting in softer doughs, reduced the staling rate for buckwheat and teff breads and increased the cell volume in sorghum and teff breads, but they did not prolong the shelf life and did not improve the aroma of breads.

Formation of EPS is a positive characteristic of sourdough LAB, since this feature influences the viscosity of sourdough (Vogel *et al.* 2002). Homopolysaccharides (HoPS) are generally applied to improve the structural characteristics of baked goods (Corsetti and Settanni 2007). Sourdough lactobacilli have not been found to produce heteropolysaccharides (HePS; Tieking and Gänzle 2005), which are mainly applied in fermented milk products (Laws and Marshall 2001). However, some authors (Galle *et al.* 2011) have assessed that the utilization of LAB strains producing HePS expands the variety of cultures and the diversity of polysaccharides for applications in gluten-free baking. For this purpose, two LAB, one *Lb. casei* and one *Lactobacillus buchneri*, were tested in sorghum sourdough and the resistance to deformation of the sorghum sourdough started with *Lb. buchneri* was registered at lower levels, due to the presence of HePS. Rühmkorf *et al.* (2012b) evaluated four EPS-producing LAB strains. One *Lb. curvatus* provided the best results, in terms of reduced bake loss, higher crumb moisture content and slower rate of staling, when used in a rice/buckwheat bread formulation (Rühmkorf *et al.* 2012b). Different strains of *Lb. sanfranciscensis*, *Lb. curvatus*, *Lactobacillus reuteri*, *Lactobacillus animalis* and *We. cibaria* did not increase the loaf volume of buckwheat, rice and sorghum sourdough breads (Galle *et al.* 2012; Rühmkorf *et al.* 2012b). Moroni *et al.* (2011b) reported that a multiple LAB strain starter culture comprising species *Lb. brevis*, *Lactobacillus paralimentarius*, *Lb. plantarum* and *We. cibaria* determined a small loaf volume of a buckwheat formulation as a result of a decrease in CO<sub>2</sub> production due to the sourdough inclusion.

From the works cited it emerged that, although the application of sourdough might not necessarily lead to improved bread quality, the effect of its inclusion in gluten-free breadmaking is strictly dependent on the flour matrix used (Wolter *et al.* 2014b).

### Microbial strategies to reduce gluten content

Gluten-free breads are undoubtedly characterized by lower-quality properties than wheat breads. For this reason, several research groups have studied different strategies to reduce the toxic effects of wheat breads for CS patients. Shan *et al.* (2002) proposed oral therapy with a prolyl-endopeptidase produced by *Flavobacterium meningosepticum* that hydrolyses the 33-mer peptide,



reported as one of the most potent peptides involved in triggering CS. This enzyme has also been purified from *Myxococcus xanthus* (Gass *et al.* 2005), *Sphingomonas capsulata* (Shan *et al.* 2004) and *Lactobacillus helveticus* (Chen *et al.* 2003). Stepniak *et al.* (2006) proposed oral supplementation with the prolyl-endopeptidase from *Aspergillus niger* that is stable under gastric conditions. However, some authors found that different LAB can decrease the CS-inducing effects of gluten.

The most significant developments in this field date to the beginning of the 2000s. Di Cagno *et al.* (2002) demonstrated active hydrolysis of various proline-rich peptides by lactobacilli. Following this finding, sourdoughs were prepared from a mixture of wheat (30%) and gluten-free (oat, buckwheat and millet; 70%) flours inoculated with strains of *Lb. alimentarius*, *Lb. brevis*, *Lb. sanfranciscensis* and *Lactobacillus hilgardii*. After 24 hours of fermentation, the gliadin fractions of the resulting bread were almost completely hydrolysed and the product was tolerated by CS patients (Di Cagno *et al.* 2004). The probiotic preparation VSL#3 (VSL Pharmaceuticals, Gaithersburg, MD, USA) containing *Streptococcus thermophilus*, *Lb. plantarum*, *Lactobacillus acidophilus*, *Lb. casei*, *Lactobacillus delbrueckii* spp. *bulgaricus*, *Bifidobacterium breve*, *Bifidobacterium longum* and *Bifidobacterium infantis* was also found to hydrolyse gliadin polipeptides (De Angelis *et al.* 2006). The same behaviour was registered for *Ent. faecalis* isolated from fermented wheat doughs (M'hir *et al.* 2008) and Wieser *et al.* (2008) confirmed the degradation of gluten proteins during sourdough fermentation in the presence of lactobacilli and enterococci during the selection of gluten-degrading LAB. Gerez *et al.* (2008) reported the functionality of LAB peptidase activities in the hydrolysis of gliadin-like fragments. In that study, none of the LAB strains alone could hydrolyse 57–89  $\alpha$ -gliadin peptide, while the combination of *Lb. plantarum* and *Pe. pentosaceus* strains led to the hydrolysis of 57% of the peptide in 8 hours.

Rizzello *et al.* (2007) showed that fermentation by selected sourdough lactobacilli and addition of fungal proteases decreased the residual concentration of gluten of wheat flour below the threshold level indicated by the Codex Alimentarius Commissions of the World Health Organization and the Food and Agricultural Organization for gluten-free foods. Thus, the application of this combined approach based on the activities of sourdough lactobacilli and fungal proteases allowed the production of baked goods made from wheat flour that were not toxic to patients with CS (Greco *et al.* 2011). M'hir *et al.* (2009) used this approach, including a pool of selected enterococci and fungal proteases. However, some limitations to the use of sourdough to reduce the intolerance of CD patients derive from the long fermentation time required for complete hydrolysis of the toxic peptides. Under such conditions, stability and dough resistance are decreased as a result of the disruption of the gluten network (Cabrera-Chávez and Calderón de la Barca 2010). Rizzello *et al.* (2014b) developed a protocol for the manufacture of a traditional wheat flour bread with an



intermediate content of gluten, enhanced digestibility, high bioavailability of free essential amino acids and high protein nutritional quality, but other studies are needed to obtain breads with complete degradation of gluten during flour fermentation and good structural and sensory features.

Thanks to the properties reported here, sourdough LAB cultures are also useful during processing of gluten-free flours because they can eliminate the risks of cross-contamination by gluten (Di Cagno *et al.* 2008).

## Conclusion

Cereal-based foods are key components of the diet of several populations. This chapter has analysed the process of fermentation of different cereal flours carried out by different microorganisms, basically LAB and yeasts, whose activities are responsible for the desirable and typical characteristics of the final products. Sourdough technology is applied worldwide to produce breads and provides a useful strategy to solve the main problems related to special bread production. Fortified and/or gluten-free breads are made with mixtures of ingredients and flours that do not generally result in high-quality products. The use of sourdough might improve several quality characteristics, but the effect depends on the active strains. From this standpoint, the selection of an ad hoc starter culture is defining to improve the sensory notes of a given fermented cereal-based product. Several LAB showed the potential to hydrolyse the toxic peptides responsible for CS during long fermentation, but the complete suitability of sourdough for the production of gluten-free breads from wheat or other flours containing gluten is still under study, in order to guarantee the safety of consumers as well as to decrease the time necessary for production.

## References

- Alfonzo, A., Ventimiglia, G., Corona, O. *et al.* (2013) Diversity and technological potential of lactic acid bacteria of wheat flours. *Food Microbiology*, **36**, 343–354.
- Allen, L.H., de Benoist, B., Dary, O. and Hurrell, R. (2006) Guidelines on Food Fortification with Micronutrients, Geneva: World Health Organization/Food and Agricultural Organization of the United Nations.
- Arendt, E.K., Moroni, A. and Zannini, E. (2011) Medical nutrition therapy: Use of sourdough lactic acid bacteria as a cell factory for delivering functional biomolecules and food ingredients in gluten free bread. *Microbial Cell Factories*, **10**, S15.
- Berghofer, L.K., Hocking, A.D., Miskelly, D. and Jansson, E. (2003) Microbiology of wheat and flour milling in Australia. *International Journal of Food Microbiology*, **85**, 137–149.
- Billings, T. (1998) On fermented foods, *Living and Fermented Foods*, <http://www.living-foods.com/articles/fermented.html> (accessed 3 September 2014).
- Blandino, A., Al-Aseeri, M.E., Pandiella, S.S., Canterob, D. and Webb, C. (2003) Cereal based fermented foods and beverages. *Food Research International*, **36**, 527–543.



- Böcker, G., Stolz, P. and Hammes, W.P. (1995) Neue Erkenntnisse zum Ökosystem Sauerteig und zur Physiologie des Sauerteig-Typischen Stämme *Lactobacillus sanfrancisco* und *Lactobacillus pontis*. *Getreide Mehl und Brot*, **49**, 370–374.
- Bottega, G., Pagani, M.A. and Mariotti, E. (2010) Tecnologia dei prodotti lievitati da forno. In M. Gobbetti and A. Corsetti (eds), *Biotechnologia dei prodotti lievitati da forno* (pp. 255–287), Milan: Casa Editrice Ambrosiana.
- Bushuk, W. (2001) Rye production and uses worldwide. *Cereal Foods World*, **46**, 70–73.
- Cabrera-Chávez, F. and Calderón de la Barca, A.M. (2010) Trends in wheat technology and modification of gluten proteins for dietary treatment of coeliac disease patients. *Journal of Cereal Science*, **52**, 337–341.
- Campbell-Platt, G. (1994) Fermented foods: A world perspective. *Food Research International*, **27**, 253–257.
- Cannella, C., Galterio, G. and Gobbetti, M. (2010) Aspetti nutrizionali dei prodotti lievitati da forno. In M. Gobbetti and A. Corsetti (eds), *Biotechnologia dei prodotti lievitati da forno* (pp. 285–295), Milan: Casa Editrice Ambrosiana.
- Cannella, C. and Piredda, M. (2006) I cereali nella dieta mediterranea. *Informatore Agrario*, **12**, 7–8.
- Caputo, I., Lepretti, M., Martucciello, S. and Esposito, C. (2010) Enzymatic strategies to detoxify gluten: Implications for celiac disease. *Enzyme Research*, **2010**, 174354.
- Carraro, L., Maifreni, M., Bartolomeoli, I. et al. (2011) Comparison of culture-dependent and -independent methods for bacterial community monitoring during Montasio cheese manufacturing. *Research in Microbiology*, **162**, 231–239.
- Catassi, C. and Francavilla, R. (2010) Intolleranza al glutine. In M. Gobbetti and A. Corsetti (eds), *Biotechnologia dei prodotti lievitati da forno* (pp. 297–316), Milan: Casa Editrice Ambrosiana.
- Chavan, J.K. and Kadam, S.S. (1989) Critical reviews in food science and nutrition. *Food Science*, **28**, 348–400.
- Chen, Y.S., Christensen, J.E., Broadbent, J.R. and Steele, J.L. (2003) Identification and characterization of *Lactobacillus helveticus* PepO2, an endopeptidase with post-proline specificity. *Applied and Environmental Microbiology*, **69**, 1276–1282.
- Coda, R., Rizzello, C.G. and Gobbetti, M. (2010) Use of sourdough fermentation and pseudo-cereals and leguminous flours for the making of a functional bread enriched of  $\gamma$ -aminobutyric acid (GABA). *International Journal of Food Microbiology*, **137**, 236–245.
- Corsetti, A., De Angelis, M., Dellaglio, F. et al. (2003) Characterization of sourdough lactic acid bacteria based on genotypic and cell-wall protein analyses. *Journal of Applied Microbiology*, **94**, 641–654.
- Corsetti, A., Gobbetti, M. and Smacchi, E. (1996) Antibacterial activity of sourdough lactic acid bacteria: Isolation of a bacteriocin-like inhibitory substance from *Lactobacillus sanfranciscensis* C57. *Food Microbiology*, **13**, 447–456.
- Corsetti, A., Lavermicocca, P., Morea, M., Baruzzi, F., Tosti, N. and Gobbetti, M. (2001) Phenotypic and molecular identification and clustering of lactic acid bacteria and yeasts from wheat (species *Triticum durum* and *Triticum aestivum*) sourdoughs of Southern Italy. *International Journal of Food Microbiology*, **64**, 95–104.
- Corsetti, A. and Settanni, L. (2007) Lactobacilli in sourdough fermentation: A review. *Food Research International*, **40**, 539–558.
- Corsetti, A., Settanni, L., Chaves-López, C., Felis, G.E., Mastrangelo, M. and Suzzi, G. (2007a) A taxonomic survey of lactic acid bacteria isolated from wheat (*Triticum durum*) and non-conventional flours. *Systematic and Applied Microbiology*, **30**, 561–571.
- Corsetti, A., Settanni, L., Valmorri, S., Mastrangelo, M. and Suzzi, G. (2007b) Identification of subdominant sourdough lactic acid bacteria and their evolution during laboratory-scale fermentations. *Food Microbiology*, **24**, 592–600.



- Czerny, M. and Schieberle, P. (2002) Important aroma compounds in freshly ground wholemeal and white wheat flour: Identification and quantitative changes during sourdough fermentation. *Journal of Agricultural and Food Chemistry*, **50**, 6835–6840.
- Dahlberg, J. (2007). Grains in human evolution. In K.D. Gifford and S. Baer-Sinnot (eds), *The Oldways Table*, Berkeley, CA: Ten Speed Press.
- De Angelis, M., Damiano, N., Rizzello, C.G., Cassone, A., Di Cagno, R. and Gobbetti, M. (2009) Sourdough fermentation as a tool for the manufacture of low-glycemic index white wheat bread enriched in dietary fibre. *European Food Research and Technology*, **229**, 593–601.
- De Angelis, M.D. and Di Cagno, R. (2010) I prodotti lievitati da forno senza glutine. In M. Gobbetti and A. Corsetti (eds), *Biotecnologia dei prodotti lievitati da forno* (pp. 317–333), Milan: Casa Editrice Ambrosiana.
- De Angelis, M., Gallo, G., Corbo, M.R. *et al.* (2003) Phytase activity in sourdough lactic acid bacteria: Purification and characterization of a phytase from *Lactobacillus sanfranciscensis* CB1. *International Journal of Food Microbiology*, **87**, 259–270.
- De Angelis, M., Rizzello, C.G., Alfonsi, G. *et al.* (2007) Use of sourdough lactobacilli and oat fibre to decrease the glycaemic index of white wheat bread. *British Journal of Nutrition*, **98**, 1196–1205.
- De Angelis, M., Rizzello, C.G., Fasano, A. *et al.* (2006) VSL#3 probiotic preparation has the capacity to hydrolyze gliadin polypeptides responsible for celiac sprue. *Biochimica et Biophysica Acta*, **1762**, 80–93.
- De Vuyst, L. and Neysens, P. (2005) The sourdough microflora: Biodiversity and metabolic interactions. *Trends in Food Science and Technology*, **16**, 43–56.
- De Vuyst, L., Vrancken, G., Ravyts, F., Rimaux, T. and Weckx, S. (2009) Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota. *Food Microbiology*, **26**, 666–675.
- Dewar, D.H., Amato, M., Ellis, H.J., Pollock, E.L. and Gonzalez-Cinca, N. (2006) The toxicity of high molecular weight glutenin subunits of wheat to patients with coeliac disease. *European Journal of Gastroenterology and Hepatology*, **18**, 483–491.
- Dhinda, F., Lakshmi, J.A., Prakash, J. and Dasappa, I. (2011) Effect of ingredients on rheological, nutritional and quality characteristics of high protein, high fibre and low carbohydrate bread. *Food Bioprocess and Technology*, **5**, 2998–3006.
- Di Cagno, R., De Angelis, M., Auricchio, S. *et al.* (2004) Sourdough bread made from wheat and nontoxic flours and started with selected lactobacilli is tolerated in celiac sprue patients. *Applied and Environmental Microbiology*, **70**, 1088–1096.
- Di Cagno, R., De Angelis, M., Calasso, M. *et al.* (2010) Quorum sensing in sourdough *Lactobacillus plantarum* DC400: Induction of plantaricin A (PlnA) under co-cultivation with other lactic acid bacteria and effect of PlnA on bacterial and Caco-2 cells. *Proteomics*, **10**, 2175–2190.
- Di Cagno, R., De Angelis, M., Lavermicocca, P. *et al.* (2002) Proteolysis by sourdough lactic acid bacteria: Effects on wheat flour protein fractions and gliadin peptides involved in human cereal intolerance. *Applied and Environmental Microbiology*, **68**, 623–633.
- Di Cagno, R., De Angelis, M., Limitone, A. *et al.* (2007) Cell-cell communication in sourdough lactic acid bacteria: A proteomic study in *Lactobacillus sanfranciscensis* CB1. *Proteomics*, **7**, 2430–2446.
- Di Cagno, R., Rizzello, C.G., De Angelis, M. *et al.* (2008) Use of selected sourdough strains of *Lactobacillus* for removing gluten and enhancing the nutritional properties of gluten-free bread. *Journal of Food Protection*, **71**, 1491–1495.
- Di Giandomenico, M. (2010) Storia e sociologia dei prodotti lievitati da forno. In M. Gobbetti and A. Corsetti (eds), *Biotecnologia dei prodotti lievitati da forno* (pp. 1–15), Milan: Casa Editrice Ambrosiana.



- Eissa, A., Hussein, A.S. and Mostafa, B.E. (2007) Rheological properties and quality evaluation of Egyptian balady bread and biscuits supplemented with flours of ungerminated and germinated legume seeds or mushroom. *Polish Journal of Food and Nutrition Sciences*, **57**, 487–496.
- Flander, L., Salmenkallio-Marttila, M., Suortti, T. and Autio, K. (2007) Optimization of ingredients and baking process for improved wholemeal oat bread quality. *LWT – Food Science Technology*, **40**, 860–870.
- Francis, F.J. (2000) *The Wiley Encyclopedia of Food Science and Technology*, Hoboken, NJ: John Wiley & Sons.
- Fuqua, W.C., Winans, S.C. and Greenberg, E.P. (1994) Quorum sensing in bacteria: The LuxR–LuxI family of cell density-responsive transcriptional regulators. *Journal of Bacteriology*, **176**, 269–275.
- Fuqua, W.C., Winans, S.C. and Greenberg, E.P. (1996) Census and consensus in bacterial ecosystems: The LuxR–LuxI family of quorum-sensing transcriptional regulators. *Annual Review of Microbiology*, **50**, 727–751.
- Galati, A., Oguntoyinbo, F.A., Moschetti, G., Crescimanno, M. and Settannia, L. (2014) The cereal market and the role of fermentation in cereal-based food production in Africa. *Food Reviews International*, **30**, 317–337.
- Gallagher, E. (2009) *Gluten-free Food Science and Technology*, Dublin: Ashtown Food Research Centre/John Wiley & Sons.
- Galle, S., Schwab, C., Arendt, E.K. and Gänzle, M.G. (2011) Structural and rheological characterisation of heteropolysaccharides produced by lactic acid bacteria in wheat and sorghum sourdough. *Food Microbiology*, **28**, 547–553.
- Galle, S., Schwab, C., Dal Bello, F., Coffey, A., Gänzle, M.G. and Arendt, E.K. (2012) Influence of *in-situ* synthesized exopolysaccharides on the quality of gluten-free sorghum sourdough bread. *International Journal of Food Microbiology*, **155**, 105–112.
- Galli, A. and Franzetti, L. (1987) Ricerche sulla composizione microbiologica della farina di grano tenero. *Annals of Microbiology*, **37**, 73–80.
- Gänzle, M.G., Vermeulen, N. and Vogel, R.F. (2007) Carbohydrate, peptide and lipid metabolism of lactic acid bacteria in sourdough. *Food Microbiology*, **24**, 128–138.
- Garofalo, C., Silvestri, G., Aquilanti, L. and Clementi, F. (2008) PCR-DGGE analysis of lactic acid bacteria and yeast dynamics during the production processes of three varieties of Panettone. *Journal of Applied Microbiology*, **105**, 243–254.
- Gass, J., Ehren, J., Strohmeier, G., Isaacs, I. and Khosla, C. (2005) Fermentation, purification, formulation, and pharmacological evaluation of a prolyl endopeptidase from *Myxococcus xanthus*: Implications for celiac sprue therapy. *Biotechnology and Bioengineering*, **92**, 674–684.
- Gerez, C.L., De Valdez, G.F. and Rollán, G.C. (2008) Functionality of lactic acid bacteria peptidase activities in the hydrolysis of gliadin-like fragments. *Letters in Applied Microbiology*, **47**, 427–432.
- Giardini, A. and Vecchietini, M. (2000). Mais o Granturco (*Zea mays* L.). In R. Baldoni and L. Giardini (eds), *Coltivazioni erbacee* (pp. 155–199), Bologna: Pàtron Editore.
- Gifford, K.D. and Baer-Sinnot, S. (eds) (2007) *The Oldways Table*, Berkeley, CA: Ten Speed Press.
- Gobbetti, M., Corsetti, A., Rossi, J., La Rosa, F. and De Vincenzi, S. (1994) Identification and clustering of lactic acid bacteria and yeasts from wheat sourdoughs of central Italy. *Italian Journal of Food Science*, **6**, 85–94.
- Gobbetti, M., Rizzello, C.G., Di Cagno, R. and De Angelis, M. (2013) How the sourdough may affect the functional features of leavened baked goods. *Food Microbiology*, **37**, 30–40.
- Gobbetti, M., Simonetti, M.S., Corsetti, A., Santinelli, F., Rossi, J. and Damiani, P. (1995) Volatile compound and organic acid production by mixed wheat sour dough starters: Influence of fermentation parameters and dynamics during baking. *Food Microbiology*, **12**, 497–507.



- Gómez, M., Oliete, B., Rosell, C.M., Pando, V. and Fernández, E. (2008) Studies on cake quality made of wheat–chickpea flour blends. *LWT – Food Science and Technology*, **41**, 1701–1709.
- Greco, L., Gobetti, M., Auricchio, R. *et al.* (2011) Safety for patients with celiac disease of baked goods made of wheat flour hydrolyzed during food processing. *Clinical Gastroenterology and Hepatology*, **9**, 24–29.
- Gullo, M., Romano, A. D., Pulvirenti, A. and Giudici, P. (2002) *Candida humilis*-dominant species in sourdoughs for the production of durum wheat bran flour bread. *International Journal of Food Microbiology*, **80**, 55–59.
- Haard, N.F., Odunfa, S.A., Lee, C.H., Quintero-Ramírez, R., Lorence-Quiñones, A. and Wachter-Radarte, C. (1999) Fermented cereals: A global perspective. *FAO Agricultural Services Bulletin*, 138.
- Hager, A.S., Axel, C. and Arendt, E.K. (2011) Status of carbohydrates and dietary fiber in gluten free diets. *Cereal Food World*, **56**, 109–114.
- Hager, A.S., Wolter, A., Czerny, M. *et al.* (2012) Investigation of product quality, sensory profile and ultrastructure of breads made from a range of commercial gluten-free flours compared to their wheat counterparts. *European Food Research and Technology*, **235**, 333–344.
- Hamad, S.H., Dieng, M.C., Ehrmann, M.A. and Vogel, R.F. (1997) Characterization of the bacterial flora of Sudanese sorghum flour and sorghum sourdough. *Journal of Applied Microbiology*, **83**, 764–770.
- Hammes, W.P., Brandt, M.J., Francis, K.L., Rosenheim, J., Seitter, M.F.H. and Vogelmann, S.A. (2005) Microbial ecology of cereal fermentations. *Trends in Food Science and Technology*, **16**, 4–11.
- Hammes, W.P. and Gänzle, M.G. (1998) Sourdough breads and related products. In B.J.B. Wood (ed.), *Microbiology of Fermented Foods* (pp. 199–216), London: Blackie Academic and Professional.
- Hammes, W.P. and Vogel, R.F. (1995) The genus *Lactobacillus*. In B.J.B. Wood and W.H. Holzappel (eds), *Genera of Lactic Acid Bacteria* (pp. 19–54), London: Blackie Academic and Professional.
- Hansen, A. and Schieberle, P. (2005) Generation of aroma compounds during sourdough fermentation: Applied and fundamental aspects. *Trends in Food Science and Technology*, **16**, 85–94.
- Harlander, S. (1992) Food biotechnology. In J. Lederberg (ed.), *Encyclopedia of Microbiology* (pp. 191–207), New York: Academic Press.
- Hausch, F., Shan, L., Santiago, N.A., Gray, G.M. and Khosla, C. (2003) Intestinal digestive resistance of immunodominant gliadin peptides. *American Journal of Physiology*, **283**, 996–1003.
- He, H. and Hoseney, R.C. (1991) Gas retention on different cereal flours. *Cereal Chemistry*, **68**, 334–336.
- Hogan, D.A. (2006) Talking to themselves: Autoregulation and quorum sensing in fungi. *Eukaryotic Cell*, **5**, 613–619.
- Iacumin, L., Cecchini, F., Manzano, M. *et al.* (2009) Description of the microflora of sourdoughs by culture-dependent and culture-independent methods. *Food Microbiology*, **26**, 128–135.
- INSOR (Istituto Italiano di Sociologia Rurale) (2012) *Atlante dei prodotti tipici: Il pane*, Rome: AGRA-RAI-ERI Editrice.
- Jenson, I. (1998) Bread and baker's yeast. In B.J.B. Wood (ed.), *Microbiology of Fermented Foods* (pp. 172–198), London: Blackie Academic and Professional.
- Kadam, M.L., Salve, R.V., Mehrajatema, Z.M. and More, S.G. (2012) Development and evaluation of composite flour for Missi roti/chapatti. *Food Processing and Technology*, **3**, 1000134.
- Kamaljit, K., Baljeet, S. and Amarjeet, K. (2010) Preparation of bakery products by incorporating pea flour as a functional ingredient. *American Journal of Food Technology*, **5**, 130–135.
- Kohajdová, Z., Karovičová, J. and Magala, M. (2011) Utilisation of chickpea flour for cracker production. *Acta Chimica Slovaca*, **4**, 98–107.



- Kohajdová, Z., Karovičová, J. and Magala, M. (2013) Effect of lentil and bean flours on rheological and baking properties of wheat dough. *Chemical Papers*, **67**, 398–407.
- Kumar, S., Tamura, K. and Nei, M. (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 150–163.
- Laws, A. and Marshall, V.M. (2001) The relevance of exopolysaccharides to the rheological properties in milk fermented with ropy strains of lactic acid bacteria. *International Dairy Journal*, **11**, 709–721.
- Lorenz, K. and Coulter, L. (1991) Quinoa flour in baked products. *Plant Foods for Human Nutrition*, **41**, 213–223.
- M'hir, S., Aldric, J.M., El-Mejdoub, T. *et al.* (2008) Proteolytic breakdown of gliadin by *Enterococcus faecalis* isolated from Tunisian fermented dough. *World Journal of Microbiology and Biotechnology*, **24**, 2775–2781.
- M'hir, S., Rizzello, C.G., Di Cagno, R., Cassone, A. and Hamdi, M. (2009) Use of selected enterococci and *Rhizopus oryzae* proteases to hydrolyse wheat proteins responsible for celiac disease. *Journal of Applied Microbiology*, **106**, 421–431.
- Macrae, R., Robinson, R.H. and Sadler, M.J. (eds) (1993) *Encyclopedia of Food Science, Food Technology and Nutrition*, London: Academic Press.
- MacRitchie, F. (1992) Physicochemical properties of wheat proteins in relation to functionality. *Advances in Food Nutritional Research*, **36**, 71–76.
- Maninder, K., Sandhu, K.S. and Singh, N. (2007) Comparative study of the functional, thermal and pasting properties of flours from different field pea (*Pisum sativum* L.) and pigeon pea (*Cajanus cajan* L.) cultivars. *Food Chemistry*, **104**, 259–267.
- Mariotti, M., Lucisano, M. and Pagani, M.A. (2006) Development of a baking procedure for the production of oat supplemented wheat bread. *International Journal of Food Science Technology*, **41**, 151–157.
- Mariotti, M., Lucisano, M., Pagani, M.A. and Iametti, S. (2008) Macromolecular interactions and rheological properties of buckwheat dough obtained from differently processed grain. *Journal Agricultural and Food Chemistry*, **56**, 4258–4267.
- McCrary, M.A., Hamaker, B.R., Lovejoy, J.C. and Eichelsdorfer, P.E. (2010) Pulse consumption, satiety, and weight management. *Advances in Nutrition*, **1**, 17–30.
- Mensah, P. (1997) Fermentation: The key to food safety assurance in Africa. *Food Control*, **8**, 271–278.
- Meroth, C.B., Hammes, W.P. and Hertel, C. (2004) Characterisation of the microbiota of rice sourdoughs and description of *Lactobacillus spicheri* sp. nov. *Systematic and Applied Microbiology*, **27**, 151–159.
- Minervini, F., De Angelis, M., Di Cagno, R. and Gobbetti, M. (2014) Ecological parameters influencing microbial diversity and stability of traditional sourdough. *International Journal of Food Microbiology*, **171**, 136–146.
- Mohamed, L., Zakaria, M., Ali, A. *et al.* (2007) Optimization of growth and extracellular glucoamylase production by *Candida famata* isolate. *African Journal of Biotechnology*, **22**, 2590–2595.
- Mohammed, I., Ahmed, A.R. and Senge, B. (2012) Dough rheology and bread quality of wheat–chickpea flour blends. *Industrial Crops and Products*, **36**, 196–202.
- Moore, M.M., Bello, F.D. and Arendt, E.K. (2008) Sourdough fermented by *Lactobacillus plantarum* FST 1.7 improves the quality and shelf life of gluten-free bread. *European Food Research and Technology*, **226**, 1309–1316.
- Moore, M.M., Juga, B., Schober, T.J. and Arendt, E.K. (2007) Effect of lactic acid bacteria on properties of gluten-free sourdoughs, batters, and quality and ultrastructure of gluten-free bread. *Cereal Chemistry*, **84**, 357–364.
- Moroni, A.V., Arendt, E.K. and Bello, F.D. (2011a) Biodiversity of lactic acid bacteria and yeasts in spontaneously-fermented buckwheat and teff sourdoughs. *Food Microbiology*, **28**, 497–502.



- Moroni, A.V., Bello, F.D., Zannini, E. and Arendt, E.K. (2011b) Impact of sourdough on buckwheat flour, batter and bread: Biochemical, rheological and textural insights. *Journal of Cereal Science*, **54**, 195–202.
- Mosca, G. and Toniolo, L. (2000) Orzo (*Hordeum* sp. pl.). In R. Baldoni and L. Giardini (eds), *Coltivazioni erbacee*, Bologna: Pàtron Editore.
- Neumann, H. and Bruemmer, J.M. (1997) Investigations with the production of gluten free bread and roll specialities. *Getreide Mehl und Brot*, **51**, 50–55.
- Nsimba, R.Y., Kikuzaki, H. and Konishi, Y. (2008) Antioxidant activity of various extracts fractions of *Chenopodium quinoa* and *Amaranthus* spp. seeds. *Food Chemistry*, **106**, 760–766.
- O'Shea, N., Arendt, E. and Gallagher, E. (2014). State of the art in gluten-free research. *Journal of Food Science*, **79**, R1067–R1076.
- Ottogalli, G., Galli, A. and Foschino, R. (1996) Italian bakery products obtained with sour dough: Characterization of the typical microflora. *Advances in Food Science*, **18**, 131–144.
- Owczarek, L., Jasińska, U., Osińska, M. and Skapska, S. (2004) Juices and beverages with a controlled phenol content and antioxidant capacity. *Polish Food Nutritional Sciences*, **5**, 261–268.
- Pagani, M.A., Lucisano, M. and Mariotti, M. (2006) Italian bakery products. In Y.H. Huy, H. Corke, I. De Leyn, W.K. Nip and N. Cross (eds), *Baking Science and Technology* (pp. 527–560), Ames, IA: Blackwell.
- Parsek, M.R. and Greenberg, E.P. (2005) Sociomicrobiology: The connections between quorum sensing and biofilms. *Trends in Microbiology*, **13**, 27–33.
- Perricone, M., Bevilacqua, A., Corbo, M.R. and Sinigaglia, M. (2014) Technological characterization and probiotic traits of yeasts isolated from Altamura sourdough to select promising microorganisms as functional starter cultures for cereal-based products. *Food Microbiology*, **38**, 26–35.
- Pogna, N.E., Tusa, P. and Boggini, G. (1996) Genetic and biochemical aspects of dough quality in wheat. *Advances in Food Science*, **18**, 145–151.
- Poutanen, K., Flander, L. and Katina, K. (2009) Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology*, **26**, 693–699.
- Prescott, L.M., Harley, J.P. and Klein, D.A. (eds) (2005) *Microbiology*, New York: McGraw-Hill.
- Raffo, A., Pasqualone, A., Sinesio, F., Paoletti, F., Quaglia, G. and Simeone, R. (2003) Influence of durum wheat cultivar on the sensory profile and staling rate of Altamura bread. *European Food Research and Technology*, **218**, 49–55.
- Rizzello, C.G., Calasso, M., Campanella, D., De Angelis, M. and Gobbetti, M. (2014a) Use of sourdough fermentation and mixture of wheat, chickpea, lentil and bean flours for enhancing the nutritional, texture and sensory characteristics of white bread. *International Journal of Food Microbiology*, **180**, 78–87.
- Rizzello, C.G., Coda, R., Mazzacane, F., Minervini, D. and Gobbetti, M. (2012). Micronized by-products from debranned durum wheat and sourdough fermentation enhanced the nutritional, textural and sensory features of bread. *Food Research International*, **46**, 304–313.
- Rizzello, C.G., Curiel, J.A., Nionelli, L. *et al.* (2014b) Use of fungal proteases and selected sourdough lactic acid bacteria for making wheat bread with an intermediate content of gluten. *Food Microbiology*, **37**, 59–68.
- Rizzello, C.G., De Angelis, M., Di Cagno, R. *et al.* (2007) Highly efficient gluten degradation by lactobacilli and fungal proteases during food processing: New perspectives for celiac disease. *Applied and Environmental Microbiology*, **73**, 4499–4507.
- Rocha, J.M. and Malcata, F.X. (1999) On the microbiological profile of traditional Portuguese sourdough. *Journal of Food Protection*, **62**, 1416–1429.
- Rossi, J. (1996) The yeasts in sourdough. *Advances in Food Science*, **18**, 201–211.
- Rühmkorf, C., Jungkunz, S., Wagner, M. and Vogel, R.F. (2012a) Optimization of homoexopolysaccharide formation by lactobacilli in gluten-free sourdoughs. *Food Microbiology*, **32**, 286–294.



- Rühmkorf, C., Rübsam, H., Becker, T. *et al.* (2012b) Effect of structurally different microbial homoexopolysaccharides on the quality of gluten-free bread. *European Food Research and Technology*, **235**, 139–146.
- Ryan, L.A.M., Dal Bello, F. and Arendt, E.K. (2008) The use of sourdough fermented by antifungal LAB to reduce the amount of calcium propionate in bread. *International Journal of Food Microbiology*, **125**, 274–278.
- Sadowska, J., Blaszcak, W. and Fornal, J. (2003) Changes of wheat dough and bread quality and structure as a result of germinated pea flour addition. *European Food Research and Technology*, **216**, 46–50.
- Salovaara, H. (1998) Lactic acid bacteria in cereal-based products. In S. Salminen and A. von Wright (eds), *Lactic Acid Bacteria Microbiology and Functional Aspects* (pp. 115–138), New York: Marcel Dekker.
- Schneider, A.V.C. (2002) Overview of the market and consumption of pulses in Europe. *British Journal of Nutrition*, **88**, S243–S250.
- Schober, T.J. and Bean, S.R. (2008) Sorghum and maize. In E. Arendt (ed.), *Gluten-Free Cereal Products and Beverages* (pp. 101–118), Food Science and Technology International Series, Amsterdam: Elsevier.
- Schober, T.J., O'Brien, C.M., McCarthy, D., Darnedde, A. and Arendt, E.K. (2003) Influence of gluten-free flour mixes and fat powders on the quality of gluten-free biscuits. *European Food Research and Technology*, **216**, 369–376.
- Schoenlechner, R., Scelsi, C., Mariotti, M., Lucisano, M. and Berghofer, E. (2006) Production and optimisation of bread from wheat and amaranth flour blends, World Grains Summit: Foods and Beverages, San Francisco, CA, 17–20 September.
- Settanni, L., Conterno, L. and Cavazza, A. (2008) Il microbiota lattico degli impasti acidi e delle materie prime usate in panificazione. *Tecnica Molitoria*, **59**, 631–638.
- Settanni, L., Guarcello, R., Gaglio, R. *et al.* (2014) Production, stability, gene sequencing and *in situ* anti-*Listeria* activity of mundticin KS expressed by three *Enterococcus mundtii* strains. *Food Control*, **35**, 311–322.
- Settanni, L., Massitti, O., Van Sinderen, D. and Corsetti, A. (2005) *In situ* activity of a bacteriocin-producing *Lactococcus lactis* strain. Influence on the interactions between lactic acid bacteria during sourdough fermentation. *Journal of Applied Microbiology*, **99**, 670–681.
- Settanni, L. and Moschetti, G. (2010) Biodiversity of sourdough lactic acid bacteria. In V. Rescigno and S. Maletta (eds), *Biodiversity Hotspots* (pp. 37–79), New York: Nova Science.
- Settanni, L. and Moschetti, G. (2014) New trends in technology and identity of traditional dairy and fermented meat production processes. *Trends in Food Science and Technology*, **37**, 51–58.
- Settanni, L., Ventimiglia, G., Alfonzo, A., Corona, O., Miceli, A. and Moschetti, G. (2013) An integrated technological approach to the selection of lactic acid bacteria of flour origin for sourdough production. *Food Research International*, **54**, 1569–1578.
- Shan, L., Marti, T., Sollid, L.M., Gray, G.M. and Khosla, C. (2004) Comparative biochemical analysis of three bacterial prolyl endopeptidases: Implications for celiac sprue. *Biochemistry Journal*, **383**, 311–318.
- Shan, L., Molberg, O., Parrot, I. *et al.* (2002) Structural basis for gluten intolerance in celiac sprue. *Science*, **297**, 2275–2279.
- Shewry, P.R. (2003) Improving wheat quality: The role of biotechnology. In S.P. Cauvain (ed.), *Bread Making: Improving Quality* (pp. 168–186), Cambridge: Woodhead.
- Shinohara, M., Aoyama, C., Fujiwara, K. *et al.* (2011) Microbial mineralization of organic nitrogen into nitrate to allow the use of organic fertilizer in hydroponics. *Soil Science and Plant Nutrition*, **57**, 190–203.
- Silano, M. and De Vincenzi, M. (1999) Bioactive antinutritional peptides derived from cereal prolamins: A review. *Nahrung*, **43**, 175–184.



- Simango, C. (1997) Potential use of traditional fermented foods for weaning in Zimbabwe. *Journal of Social Science and Medicine*, **44**, 1065–1068.
- Sivam, A.S., Sun-Waterhouse, D., Quek, S. and Perera, C.O. (2010) Properties of bread dough with added fiber polysaccharides and phenolic antioxidants: A review. *Journal of Food Science*, **75**, R163–R174.
- Soni, S.K. and Sandhu, D.K. (1990) Indian fermented foods: Microbiological and biochemical aspects. *Indian Journal of Microbiology*, **30**, 135–157.
- Spicher, G. (1999) Zur Geschichte des Sauerteiges. In G. Spicher and H. Stephan (eds), *Handbuch Sauerteig: Biologie, Biochemie, Technologie* (pp. 3–7), Hamburg: Behr's.
- Spiertz, J.H.J. and Ewert, F. (2009) Crop production and resource use to meet the growing demand for food, feed and fuel: Opportunities and constraints. *NJAS Wageningen Journal of Life Science*, **56**, 281–300.
- Stepniak, D., Spaenij-Dekking, L., Mitea, C. et al. (2006) Highly efficient gluten degradation with a newly identified prolyl endoprotease: Implications for celiac disease. *American Journal of Physiology – Gastrointestinal and Liver Physiology*, **291**, G621–G629.
- Stolz, P. (1999) Mikrobiologie des Sauerteiges. In G. Spicher and H. Stephan (eds), *Handbuch Sauerteig: Biologie, Biochemie, Technologie* (pp. 35–60), Hamburg: Behr's.
- Tack, G.J., Verbeek, W.H., Schreurs, M.W. and Mulder, C.J. (2010) The spectrum of celiac disease: Epidemiology, clinical aspects and treatment. *Nature Reviews Gastroenterology and Hepatology*, **7**, 204–213.
- Taylor, J.R.N. and Emmambux, N. (2008) Gluten-free foods and beverages from millets. In E. Arendt and F. Dal Bello (eds), *Gluten-Free Cereal Products and Beverages* (pp. 119–148), Food Science and Technology International Series, Amsterdam: Elsevier.
- Taylor, J.R.N. and Parker, M.L. (2002) Quinoa. In P.S. Belton and J.R.N. Taylor (eds), *Pseudocereals and Less Common Cereals: Grain Properties and Utilization*, Berlin: Springer.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) The CLUSTAL-X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**, 4876–4882.
- Tieking, M. and Gänzle, M.G. (2005) Exopolysaccharides from cereal-associated lactobacilli. *Trends in Food Science and Technology*, **16**, 79–84.
- Tiwari, B.K., Brennan, C.S., Jaganmohan, R., Surabi, A. and Alagusundaram, K. (2011) Utilisation of pigeon pea (*Cajanus cajan* L) by-products in biscuit manufacture. *LWT – Food Science and Technology*, **44**, 1533–1537.
- Toderi, G. (1989). Frumento (*Triticum* spp.). In R. Baldoni and L. Giardini (eds), *Coltivazioni erbacee* (pp. 15–89), Bologna: Pàtron Editore.
- Toderi, G. (1993) Aspetti generali sulle colture cerealicole. In R. Baldoni and L. Giardini (eds), *Coltivazioni erbacee* (pp. 26–42), Bologna: Pàtron Editore.
- Topping, D. (2007) Cereal complex carbohydrates and their contribution to human health. *Journal of Cereal Science*, **46**, 220–229.
- Valmorri, S., Settanni, L., Suzzi, G., Gardini, F., Vernocchi, P. and Corsetti, A. (2006) Application of a novel polyphasic approach to study the lactobacilli composition of sourdoughs from the Abruzzo region (central Italy). *Letters in Applied Microbiology*, **43**, 343–349.
- Valmorri, S., Tofalo, R., Settanni, L., Corsetti, A. and Suzzi, G. (2010) Yeast microbiota associated with spontaneous sourdough fermentations in the production of traditional wheat sourdough breads of the Abruzzo region (Italy). *Antonie Van Leeuwenhoek*, **97**, 119–129.
- Vogel, R.F., Ehrmann, M.A. and Gänzle, M. G. (2002) Development and potential of starter lactobacilli resulting from exploration of the sourdough ecosystem. *Antonie van Leeuwenhoek*, **81**, 631–638.



- Vogelmann, S.A., Seitter, M., Singer, U., Brandt, M.J. and Hertel, C. (2009) Adaptability of lactic acid bacteria and yeasts to sourdoughs prepared from cereals, pseudocereals and cassava and use of competitive strains as starters. *International Journal of Food Microbiology*, **130**, 205–212.
- Wieser, H., Vermeulen, N., Gaertner, F. and Vogel, R.F. (2008) Effects of different *Lactobacillus* and *Enterococcus* strains and chemical acidification regarding degradation of gluten proteins during sourdough fermentation. *European Food Research Technology*, **226**, 1495–1502.
- Wolter, A., Hager, A.S., Zannini, E., Czerny, M. and Arendt, E.K. (2014a) Impact of sourdough fermented with *Lactobacillus plantarum* FST 1.7 on baking and sensory properties of gluten-free breads. *European Food Research and Technology*, **239**, 1–12.
- Wolter, A., Hager, A.S., Zannini, E., Czerny, M. and Arendt, E.K. (2014b) Influence of dextran-producing *Weissella cibaria* on baking properties and sensory profile of gluten-free and wheat breads. *International Journal of Food Microbiology*, **172**, 83–91.