A REVIEW OF THE APPLICATION OF SOURDOUGH TECHNOLOGY TO WHEAT BREADS

CHARMAINE I. CLARKE*,† AND ELKE K. ARENDT*

*Department of Food and Nutritional Sciences
National University of Ireland
Cork, Ireland
†National Food Biotechnology Centre
National University of Ireland
Cork, Ireland

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I. INTRODUCTION

A. HISTORY OF SOURDOUGH TECHNOLOGY

Bread in its many forms is one of the most staple foods consumed by humans (Cauvain, 1998a). The art and craft of bread making existed at the outset of recorded history and predates it, as documented by excavations undertaken in many parts of the world (Spicer, 1975). Depictions of the activities involved in the baking of bread have been found in tomb paintings from ancient Egypt, and one of the most detailed accounts of baking dates back to the reign of Seti I (1303–1290 B.C.) (Ezzamel, 1997). The purpose of bread making is to present cereal flours to the consumer in an attractive, palatable, and digestible form (Chamberlain, 1975). The earliest breads were likely unleavened or flat (Quail, 1996), but the first major technical innovation was the introduction of leavening, which yielded breads of superior palatability (Chamberlain, 1975). Early dough fermentation would probably have relied on a mixture of naturally occurring yeasts and lactic acid bacteria (Oura *et al*., 1982; Williams and Pullen, 1998). The underlying functionality of such an adventitious microbial population is that a dough formed by the addition of water to ground cereals will with time be fermented by the microorganisms naturally present to become a sourdough characterized by acid taste, aroma, and increased volume due to gas formation (Hammes and Gänzle, 1998). The use of the sourdough process as a form of leavening is one of the oldest biotechnological processes in food production (Röcken and Voysey, 1995). To facilitate continuous production, one could save a portion of ripe sourdough dough to seed subsequent doughs, a process that continued into the nineteenth century (Williams and Pullen, 1998). In addition to the yeasts naturally present on the cereal grains, brewers' yeast was often added to enhance the fermentation process (Oura *et al*., 1982; Röcken and Voysey, 1995; Williams and Pullen, 1998), but the sourdough procedure predominated in bread making until specially prepared baker's yeast became available in the nineteenth century (Pederson, 1971).

B. USE OF SOURDOUGH IN RYE PRODUCTS

The availability of baker's yeast has not eliminated the use of sourdoughs in rye bread making in which a reduction in pH is necessary to achieve suitability for baking (Hammes and Gänzle, 1998; Oura *et al*., 1982; Salovaara, 1998). This results from the inability of rye doughs to form a gluten network, which in wheat doughs provides the water-binding and gas-retaining properties. In rye, these functions are taken over by pentosans, whose solubility and swelling increase with a decrease in pH (Hammes and

Gänzle, 1998). Sour conditions also partially inactivate the increased enzyme activity in rye flour, particularly amylase activity (Seibel and Brümmer, 1991). This is an important aspect, because the starch in rye gelatinizes at a relatively low temperature, 55–70 °C, which coincides with the temperature range for maximal α-amylase activity (Cauvain, 1998b). An excessive amount of α-amylase in rye flour produces not only a sticky crumb, but at higher levels, a very open grain, a reduction in loaf volume, and in some instances, cavitation of the loaf (Reed, 1966). The acidification also exerts positive effects on the structure of starch granules, leading to increased water-binding capacity (Hammes and Gänzle, 1998). Acidification of rye doughs improves their physical properties by making them more elastic and extensible and confers the acid flavor notes so characteristic of rye breads.

C. USE OF SOURDOUGH IN WHEAT PRODUCTS

Whereas sourdough is an essential ingredient for ensuring baking properties of doughs containing more than 20% rye flour, its addition to wheat doughs remains optional (Röcken, 1996). However, a vast array of traditional products rely on the use of sourdough fermentation to yield baked goods with particular quality characteristics. Some examples include the well-known Italian products associated with Christmas, Panettone, which originated in Milan (Sugihara, 1977), and Pandoro originally from Verona (Zorzanello and Sugihara, 1982) or their counterpart, Colomba, which is traditionally associated with Easter (Sugihara, 1977). San Francisco sourdough French breads (Kline *et al*., 1970) and soda crackers (Sugihara, 1985) are other examples of wheat products that rely on the process of souring. The same process is also used in the production of a number of flat breads, a typical example of which is the Egyptian baladi bread (Qarooni, 1996). Further to these traditional varieties of baked goods, the use of lactic acid bacteria and yeasts in the form of sourdough is well established in Italy (Corsetti *et al*., 2001), Germany (Seibel and Brümmer, 1991), Spain (Barber and Báguena, 1989a), and France (Infantes and Tourner, 1991). The use of sourdough in wheat breads has gained popularity as a means to improve the quality and flavor of wheat breads (Brümmer and Lorenz, 1991; Corsetti *et al*., 2000; Stear, 1990; Thiele *et al*., 2002).

II. MICROFLORA OF SOURDOUGH

By one definition, sourdough has been described as "a dough made of cereal products (and other ingredients, if required), liquids, and microorganisms (such as lactic acid bacteria and yeasts) in an active state. Acidification

(fermentation) produced by these substances is a continuous process. The activity of the microorganisms is never interrupted. Microorganisms contained in the flour can also be activated in the course of this process" (Seibel and Brümmer, 1991). It is considered that the occurrence of lactic acid bacteria and yeasts in sourdoughs and the association between acidification and bacterial metabolism was first demonstrated in 1894 (Hammes and Gänzle, 1998). Numerous species of lactic acid bacteria occur naturally in wheat flour, including members of the genera *Lactobacillus*, *Pediococcus*, *Enterococcus*, *Lactococcus*, and *Leuconostoc* (Hammes and Vogel, 1997). Likewise, numerous species of lactic acid bacteria, mainly belonging to the genus *Lactobacillus*, have been isolated from sourdoughs (Corsetti *et al*., 2001; Ottogalli *et al*., 1996). Lactobacilli are gram-positive, non–spore-forming rods or coccobacilli with complex nutritional requirements. They are found where rich carbohydrate-containing substrates are available such as plants or material of plant origin and in human-made habitats such as that of fermenting food (Hammes and Vogel, 1995). Their optimum growth temperature is 30–40 °C (Bergey, 1994). Lactic acid is the principal product of carbohydrate fermentation by sourdough lactic acid bacteria (Seibel and Brümmer, 1991). Different species, however, can vary greatly in the manner in which they can metabolize carbohydrates and thus may be broadly classified as those that produce lactic acid as the sole (homofermentative) or as a major (heterofermentative) end product of fermentation. Considerable amounts of acetic acid are also formed by those heterofermentative species (Oura *et al*., 1982).

From a microbiological point of view, the definition of sourdough given earlier in this chapter makes reference not only to the presence of lactic acid bacteria but also to the presence of yeasts. Associations of yeasts and lactic acid bacteria are often encountered or used in the production of beverages and fermented foods (Gobbetti, 1998). The vast majority of yeasts found in sourdoughs have been allotted to the species *Candida milleri, Candida holmii, Saccharomyces exiguus*, and *Saccharomyces cerevisiae* (Hammes and Gänzle, 1998). There are many trophic and nontrophic interactions between the associations of lactic acid bacteria and yeasts found in sourdough (Gobbetti, 1998).

A. SOURCES OF LACTIC ACID BACTERIA FOR SOURDOUGH

Reliance on the fortuitous microorganisms of flour to "spontaneously start" the fermentation process is probably the oldest method used for sourdough production (Spicher, 1983). These organisms may originate from the cereal itself, from contaminants of baker's yeast, or from the milling or baking environment (Hammes and Gänzle, 1998). In this context, a dough prepared

from equal quantities of flour and water allowed to stand for 24 hours at 26–35 °C will begin to ferment when gram-negative enteric bacteria present in the flour initiate the process. With repeated additions of flour and water, the dough will become more acidic as the microflora becomes dominated by lactic acid bacteria (Röcken and Voysey, 1995). It is evident from sourdoughs that have been propagated for some time that selection occurs during propagation, leading to the establishment of, usually, one or two species at numbers three or four orders of magnitude above those of the adventitious microbial flora (Hammes and Gänzle, 1998; Hammes *et al.*, 1996; Meroth *et al.*, 2003). The addition of a portion of ripe sour, for example, seed sour, from a previous batch and continuous propagation of the same is another method by which a sourdough may be started (Spicher, 1983). To minimize the variations between sourdoughs where the composition of the microflora is not critically controlled, the application of defined starter cultures for sourdough production has been developed. The commercial availability of single and multiple strain preparations of lactic acid bacteria means that continuous propagation is not necessary and that a high standard of bread quality can be consistently maintained (Hammes, 1990).

B. CLASSIFICATION OF SOURDOUGHS

Based on the technology applied for their production, sourdoughs have been classified into three groups (Böcker *et al.*, 1995). Most traditional sourdoughs can be classified as type I doughs. Doughs of this type are characterized by continuous propagation to maintain the activity of the microflora, and this is typically achieved by the use of a multistage process. *Lactobacillus sanfranciscensis* is the dominant organism isolated from these sourdoughs of this type, and *Lactobacillus pontis* may also be found. The organisms occurring in doughs of this type are sensitive to low pH levels, so if the sourdough is maintained at ambient temperature and acidification continues, more acid-resistant species will become dominant.

In keeping with the requirements of modern baking technology, more efficient fermentation processes are emerging within the field of sourdough applications. Type II sourdoughs are those that are produced by continuous propagation and extended fermentation times. This type of sourdough fermentation originates from the demand for continuous production of pumpable sourdoughs in industrial applications in bread factories, bakeries, and producers of sourdough products (Meuser *et al.*, 1987). Type II doughs can be produced in large volumes and stored for up to 1 week. In contrast to type I doughs, type II sourdoughs exhibit higher dough yields, that is, softer and increased fermentation temperature. In view of the fact that a single

fermentation period of 15–20 hours is employed, gas formation by the lactic acid bacteria is strongly reduced and baker's yeast must be applied to the dough for the purposes of leavening. Microorganisms found in wheat sourdoughs of this type belong to the species *L. pontis*, *Lactobacillus panis*, *Lactobacillus reuteri*, and *Lactobacillus fermentum* (Vogel *et al.*, 1999).

Type III sourdoughs can be regarded as artificially composed dried sourdoughs in that lactic acid starter bacteria have been selected with respect to their robustness for drying. They are added as a souring enhancer to sourdoughs for bread dough production. Isolates from these sourdoughs matching the desired properties can be allotted to the species *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Pediococcus pentosaceus* (Böcker *et al.*, 1995). Applying lactic acid bacteria in a freeze-dried state is another method used to initiate sourdough fermentation. Bacterial isolates from, for example, a mature sourdough or other natural environment are selected and tested for their suitability for being employed as sourdough starters and their viability after drying. Freeze-dried strains of *Lactobacillus delbrueckii*, *L. brevis*, *L. plantarum*, and *Lactobacillus fructivorans*, for example, have been described (Hammes and Gänzle, 1998). In contrast to the type I sourdough starters, these strains are not necessarily well adapted to the cereal environment, so frequent inoculation is advised (Röcken and Voysey, 1995).

III. POSITIVE EFFECTS OF SOURDOUGH ON WHEAT BREAD QUALITY

There is considerable consensus regarding the positive effects conferred on bread products by the use of sourdough. From a consumer perspective, the use of sourdough confers a natural image on the product (Salovaara, 1998). Lactic acid bacteria have a long history of use in food and are "generally regarded as safe" organisms (Magnusson *et al.*, 2003).

A. NUTRITIONAL QUALITY

It has been observed that bread containing lactic acid produced during sourdough fermentation or added directly can lower the postprandial glucose and insulin responses in humans (Liljeberg and Björck, 1994; Liljeberg *et al.*, 1995; Östman *et al.*, 2002). The presence of sourdough acids has also been reported to have a positive effect on the formation of resistant starch (Liljeberg and Björck, 1994; Liljeberg *et al.*, 1996). Furthermore, the nutritional quality of sourdough baked goods is improved with regards to mineral availability (Larsson and Sandberg, 1991; Lopez *et al.*, 2001, 2003;

Salovaara and Goransson, 1983). Phytate is present in all cereals and forms insoluble complexes with the minerals in flour, consequently reducing its bioavailability, so excessive amounts of it in the diet can have a negative effect. The low pH values associated with chemically or microbiologically acidified wheat doughs lead to solubilization of the phytate complex, thus increasing mineral bioavailability. In addition, it has been reported that exopolysaccharides produced by *L. sanfranciscensis* may improve the nutritional properties of sourdough fermented products in view of the fact that they may be metabolized by bifidobacteria (Korakli *et al.*, 2002). In addition to these nutritional advantages, sourdough technology may have the potential for the production of special sourdough type of breads with a low content of gliadin peptides toxic for those with celiac disease. This is in view of the fact that selected sourdough lactic acid bacteria have been shown to have hydrolyzing activities toward prolamin peptides involved in human cereal intolerance (Di Cagno *et al.*, 2002).

B. MICROBIOLOGICAL SPOILAGE

The general trend to reduce the use of preservatives and treatments that might affect healthy attributes of food has led to attempts to improve bread quality and shelf-life through formulation with compounds naturally occurring in foods (Barber *et al.*, 1992). There has been much interest in the potential application of lactic acid bacteria as a means of biopreservation, that is, control of one organism by another (Magnusson *et al.*, 2003). In addition to the control and inhibition of spoilage organisms during fermentation due to the low pH values (Hammes and Gänzle, 1998; Salovaara, 1998), positive effects of the use of sourdough on the mould-free shelf-life of wheat bread have been reported (Barber *et al.*, 1992; Lavermicocca *et al.*, 2000; Salovaara and Valjakka, 1987). Prevention or limitation of the growth of rope-producing spores of *Bacillus subtilis* has also been achieved through the use of sourdough or certain strains of sourdough isolates in bread (Pepe *et al.*, 2003; Röcken and Voysey, 1993; Rosenquist and Hansen, 1998). With respect to deterioration in the quality of bread during shelf life, mould growth is the most common cause of microbial spoilage. In addition to the economic losses associated with spoilage of this nature, another concern is the possibility that mycotoxins produced by the moulds may cause public health problems (Legan, 1993). Certain sourdough lactic acid bacteria and their components have been shown to have an antifungal effect against various fungal species isolated from flour and bakery products, some of which are toxin producers (Lavermicocca *et al.*, 2000, 2003). The same effect has been demonstrated in the context of sourdough wheat breads (Lavermicocca *et al.*, 2000).

It is evident that the antifungal phenomenon is not only due to the development of organic acids during the sourdough fermentation process, despite that an improvement in the shelf life of sourdough baked products was initially attributed to the production of organic acids, particularly acetic acid, by lactic acid bacteria (Röcken, 1996). Barber *et al.* (1992) reported no correlation between bread shelf-life and pH level, but these authors concluded, however, that the type and amount of acid present may have an effect on other microstatic agents. Furthermore, Corsetti *et al.* (1998b), employing an agar–well-diffusion assay, found that the individual organic acids (acetic, caproic, propionic, butyric, *n*-valeric, and formic) produced by *L. sanfrancisco* CB1 gave no halos of inhibition against *Fusarium graminearum*. These authors did, however, find that a mixture of all six organic acids had a strongly inhibitory effect. In the same vein, Magnusson *et al.* (2003) found that those lactic acid bacteria that did not exhibit an antifungal effect actually produced more lactic acid than those strains that had highly active antifungal activities. The same author (Magnusson, 2003) does, however, note that the antifungal activities of lactic acid bacteria are complex and that the presence of organic acids may indeed play a role. Other substances contributing to activity of this nature may include reuterin, hydroxy fatty acids, proteinaceous compounds, cyclic dipeptides, 3-phenyllactic acid, caproic acid, and diacetyl hydrogen peroxide (Magnusson, 2003).

There is great divergence among lactic acid bacteria in terms of their antifungal activity. One study that evaluated more than 200 strains of sourdough lactic acid bacteria, using a well-diffusion assay, reported that the antimould activity varied greatly among the strains and was mainly detected within obligately heterofermentative *Lactobacillus* species (Corsetti *et al.*, 1998b). Lavermicocca *et al.* (2000) screened a number of strains isolated from sourdough breads and also found that the rate of inhibition of a number of fungal species was highly strain dependent. These authors found that *L. plantarum* 21B, which is facultatively heterofermentative but typically homofermentative, had the greatest inhibition spectrum.

C. FLAVOR

Taste and flavor of bread can be improved with optimal use of sourdough (Seibel and Brümmer, 1991). The flavor of sourdough wheat bread is richer and more aromatic than in wheat bread, a factor that can be attributed to the long fermentation time of sourdough (Brümmer and Lorenz, 1991). Studies on the influence of lactic acid bacteria on the aroma of wheat bread revealed a positive influence, particularly on the crumb aroma (Hansen and Hansen, 1996). The concentration of 2-phenylethanol, one of the most potent odorants of wheat bread crumb (Grosch and Schieberle,

1997), was increased in sourdough-enriched bread crumb (Gassenmeier and Schieberle, 1995). It has been demonstrated that the production of volatile flavor components in sourdough is strongly dependent on the starter culture, but the role played by the flour used has also been recognized (Hansen and Hansen, 1994a). Few volatile compounds have been identified in chemically acidified doughs compared to sourdoughs (Hansen and Hansen, 1994b), and the intensity of flavor was found to be greater in breads prepared with biologically acidified preferment than in those that were chemically acidified (Thiele *et al*., 2002). The main influence of microorganisms on sourdough flavor has been identified as their ability to enhance or reduce the amount of specific volatiles already present in the flour (Czerny and Schieberle, 2002). An increase in the level of amino acids in doughs, especially ornithine, has also been associated with improved bread flavor (Thiele *et al*., 2002).

D. BREAD CHARACTERISTICS

Loaf-specific volume is a primary quality characteristic of bread (Maleki *et al*., 1980). The application of sourdough to wheat breads has a positive impact on bread volume (Barber *et al*., 1989b, 1992; Clarke *et al*., 2002; Collar *et al*., 1994a; Corsetti *et al*., 1998a, 2000; Crowley *et al*., 2002). The rate of application is important, however, because optimum levels of sourdough must be applied to achieve optimal bread quality (Barber *et al*., 1992; Collar *et al*., 1994a; Crowley *et al*., 2002). The nature of the acidification process may also be key. Regarding biological acidification, it has been reported that chemical acidification, in the absence of any considerable fermentation period, does not improve loaf-specific volume (Clarke *et al*., 2002). A chemically acidified dough fermented for more than 3 hours, however, was found to yield breads with greater volume than any of their counterparts fermented with lactic acid bacteria, a finding attributed to yeast metabolism being favored by acidic conditions (Corsetti *et al*., 2000). In keeping with the findings of Maleki *et al*. (1980) who reported that larger loaf size produced softer bread, sourdough breads have been shown to have lower crumb firmness values (Clarke *et al*., 2002; Collar, 1994a; Corsetti *et al*., 2000; Crowley *et al*., 2002). Crumb grain, described as the exposed cell structure of the crumb when a loaf of bread is sliced, is another important bread quality characteristic affected by the addition of sourdough. It is generally acknowledged that holes of relatively small size ($\sim$1 or 2 mm) are required in bakery products, whereas large voids or irregular crumb distributions are undesirable (Cauvain, 1998a). An increase in the mean cell area, within the range that is still desirable, has been demonstrated via addition of 20% sourdough (Crowley *et al*., 2002).

E. STALING

Bread is generally viewed as a perishable commodity, which is best consumed when "fresh." The loss of perceived freshness is due to a number of factors, which may generally be categorized as those attributable to microbial spoilage and those that are due to a series of complex processes collectively known as *staling* (Pateras, 1998). *Staling* has been defined as "a term which indicates decreasing consumer acceptance of bakery products caused by changes in crumb other than those resulting from the action of spoilage organisms" (Bechtel *et al*., 1953). Despite extensive study, bread staling has not been eliminated and remains responsible for huge economic losses (Gray and Bemiller, 2003). Although a complex series of events occur during staling, including changes in the crystallinity of the starch during storage (Cauvain and Young, 2000), bread staling is mainly associated with the firming of the crumb (Gray and Bemiller, 2003; Pateras, 1998). The application of lactic acid bacteria in the form of sourdough has been reported to have positive effects on bread staling. One such effect is an improvement in loaf-specific volume, which is associated with a reduction in the rate of staling (Axford *et al*., 1968; Maleki *et al*., 1980), as has been demonstrated by a reduction in crumb softness for sourdough breads during shelf life (Clarke *et al*., 2002; Corsetti *et al*., 2000; Crowley *et al*., 2002). A decrease in the staling rate as measured by differential scanning calorimetry has also been reported for breads containing sourdough (Barber *et al*., 1992; Corsetti *et al*., 1998a, 2000). It has been noted, however, that the antistaling effect seen for sourdough is strain specific, involving dynamics other than those associated with the degree of acidification. Activities associated with bacterial hydrolysis of starch and the proteolysis of gluten subunits have been proposed (Corsetti *et al*., 1998a).

IV. UNDERSTANDING THE TECHNOLOGICAL FUNCTIONALITY OF SOURDOUGH APPLICATION

Despite its long tradition and the well-documented positive effects conferred on bread products by its use, various details about sourdough technology have not been fully understood. This remains the case not only regarding sourdough microbial ecology and physiology, despite much progress in this regard (Brandt and Hammes, 2001; Gobbetti, 1998; Hammes and Gänzle, 1998), but also regarding the influence of sourdough on the structure of dough and bread. The mechanisms at work in sourdough and its application are complex and numerous (Hammes and Gänzle, 1998). Various flour characteristics and process parameters contribute to exercising very

particular effects on the metabolic activity of the sourdough microflora. During fermentation, biochemical changes occur in the carbohydrate and protein components of the flour due to the action of microbial and indigenous enzymes. The rate and extent of these changes greatly influence the properties of the sourdough and ultimately the quality of the final baked product. A number of hypotheses have been put forward that can help explain the effects of sourdough on dough and bread quality, including those that are related to the direct impact of pH on dough structure, those corresponding to the effect of acid on cereal enzymes, and those that are related to the effect of the microorganisms alone.

A. PRIMARY EFFECTS OF ACIDIFICATION

The pH of a ripe sourdough varies with the nature of the process and starter culture used, but for wheat sourdoughs, it ranges from 3.5 to 4.3 (Clarke *et al.*, 2002; Collar *et al.*, 1994a; Thiele *et al.*, 2002; Wehrle and Arendt, 1998). The nature of the flour, in particular its ash content, has a considerable effect on acidification characteristics (Collar *et al.*, 1994b). Depending on the rate of addition, the pH of the bread dough will also vary. Given a typical application rate of approximately 20%, dough pH values ranging from 4.7 to 5.5 have been reported (Clarke *et al.*, 2002; Collar *et al.*, 1994a). The acidification of the sourdough and the partial acidification of the bread dough will no doubt have a direct impact on structure-forming components like gluten, starch, and arabinoxylans. It was reported almost a century ago (Osborne, 1907) that the presence of acid increased the solubility of the glutenin fraction extracted from wheat flour. The swelling of gluten in acid is a well-known effect (Axford *et al.*, 1979; Zeleny, 1947), and mild acid hydrolysis of starch in sourdough systems has been hypothesized (Barber *et al.*, 1992). Acids strongly influence the mixing behavior of doughs, whereby doughs with lower pH values require a slightly shorter mixing time and have less stability than normal doughs (Hoseney, 1994). The direct influence of organic acids on the rheological properties of dough has been examined intensely using both empirical (Maher Galal *et al.*, 1978; Tanaka *et al.*, 1967; Tsen, 1966; Wehrle *et al.*, 1997) and fundamental techniques (Clarke *et al.*, 2002, in press; Wehrle *et al.*, 1997).

Several studies directly focused on the influence of added organic acids and sodium chloride on rheological properties measured using the farinograph (Maher Galal *et al.*, 1978; Tanaka *et al.*, 1967; Wehrle *et al.*, 1997) and extensograph (Tanaka *et al.*, 1967; Tsen, 1966). The farinograph is commonly used to provide empirical information regarding the mixing properties of dough (Spies, 1990). The water absorption of flour, as determined using the farinograph, is an important factor influencing the handling properties and

machinability of dough in large mechanized bakeries and is related to the quality of the finished baked product (Catterall, 1998). Studies of wheat dough using the farinograph showed that water uptake or consistency was increased by added organic acids in the absence of salt (Maher Galal *et al*., 1978; Tanaka *et al*., 1967). The addition of organic acids also substantially decreased mixing time and weakened the dough (Maher Galal *et al*., 1978; Wehrle *et al*., 1997). Maher Galal *et al*. (1978) put forward the hypothesis on a molecular level, which stated that in an acidic environment, there is a sizable positive net charge and protein solubility is increased. The increased intramolecular electrostatic repulsion leads to an unfolding of the gluten proteins and an increased exposure of hydrophobic groups, but the presence of strong intermolecular electrostatic repulsive forces prevents the formation of new bonds. The net effect of these events is a weakening of the structure and thus a softening effect. Such a hypothesis is further supported by Osborne (1907) and Takeda *et al*. (2001) who reported increased solubility of the constituent gluten proteins at acidic pH values. This disentanglement of the gluten protein network upon the addition of acid is quite in keeping with the results obtained from empirical measurement of dough properties using the extensograph, which found that the addition of acid, in the presence of salt, resulted in doughs with increased resistance and decreased extensibility (Clarke *et al*., 2002; Tanaka *et al*., 1967; Tsen, 1966). An explanation of the dough response during this test, given in terms of the entangled protein network model, is that when a piece of dough is subjected to elongation, it will tear when the network between the two entangled regions reaches its full extension; thus, the more entangled the network, the higher its resistance to deformation (Masi *et al*., 2001).

Fundamental rheological studies on chemically acidified doughs have also been performed (Clarke *et al*., 2002; Wehrle *et al*., 1997). Wehrle *et al*. (1997) reported that under optimal mixing conditions, the addition of acids leads to dough with lower phase angle values and thus more elastic behavior. The phase angle ranges from 0 degrees (ideally elastic material, hookean solid) to 90 degrees (ideally viscous material, newtonian liquid). For all viscoelastic materials, the phase angle is between 0 and 90 degrees, and the lower the values, the more elastic the material. Clarke *et al*. (2002) reported that the direct addition of an organic acid slightly decreased the absolute value of the complex modulus (i.e., dough firmness). The effect of acid addition on the rheology of a sourdough preferment has also been determined at the outset of the fermentation period (Clarke *et al*., 2004) at which point the changes directly associated with acidic pH values were seen to be an increase in elasticity and a simultaneous decrease in viscosity. A fundamental rheological evaluation of the effect of acid and salt on model gluten systems

was also indicative of an increase in both the softness and the elasticity of gluten in the presence of acid (Schober *et al*., 2003).

B. SECONDARY EFFECTS OF ACIDIFICATION

In addition to the direct impact of decreasing pH values on dough characteristics, secondary effects of acidification and fermentation time may include changes in the activity of cereal or bacterial enzymes associated with changes in the pH of the environment during the fermentation period. Kawamura and Yonezawa (1982) described wheat flour proteases that have optimal activity around a pH level of 4. In addition, Bleukx *et al*. (1997) detected proteolytic enzymes with acidic pH optima in vital wheat gluten. In terms of the effects of acidic pH values on dough characteristics, Wu and Hoseney (1989) showed for cracker sponges that a pH value of 4.1 was most effective in reducing the resistance to extension during a 12-hour fermentation period. In the same vein, Thiele *et al*. (2002) found a greater increase in the concentration of particular amino acids in an acidified relative to a nonacidified dough system during a 50-hour fermentation period. These authors concluded that the most important factors governing the levels of amino acids in wheat dough were dough pH value, fermentation time, and the consumption of amino acids by the fermentative microflora. Another study, using both empirical and fundamental techniques to compare the rheological properties of chemically and biologically acidified doughs, found that the addition of biological acidified material resulted in major changes in the structure of the dough not comparable to those attributable to the presence of acid alone (Clarke *et al*., 2002). This study did not employ a fermentation period for the chemically acidified treatment and thus concluded that in contrast to the limited time frame during which enzyme activity could have an impact on the structure of the chemically acidified dough, the fermentation period of the biologically acidified treatments could allow for an extended period of enzymatic activity further enhanced by acidic pH values.

C. PROTEOLYSIS DURING SOURDOUGH FERMENTATION

The effect of proteolysis on the structure-forming components of dough is another well-documented aspect of sourdough fermentation (Corsetti *et al*., 2000; Di Cagno *et al*., 2002; Kawamura and Yonezawa, 1982). Despite that proteolysis and the proliferation of amino acids by enzymatic release during sourdough fermentation is well documented (Collar and Martinez, 1993; Collar *et al*., 1992; Di Cagno *et al*., 2002; Gobbetti *et al*., 1994; Thiele *et al*., 2002, 2003), proteolysis during sourdough fermentation remains unclear.

This is due to the nature of the sourdough system where the effects of acidification and the endogenous microbial and cereal proteases all contribute to a complex set of dynamics. It is further complicated by the fact that there may be divergence between studies in terms of the particular lactobacilli used given that proteolytic activity is strain dependent. The enhanced proteolysis seen during sourdough fermentation has been attributed to both the proteolytic activity of lactic acid bacteria and that of cereal proteases.

With regards to the bacteria, the level of proteolytic activity on wheat flour fractions has been found to be very strain dependent (Corsetti *et al*., 1998a; Di Cagno *et al*., 2002; Gobbetti *et al*., 1996). Certain bacterial strains have the specific capacity to hydrolyze albumin, globulin, and gliadin fractions of wheat flour (Di Cagno *et al*., 2002). Using free amino acid concentration as a measure of proteolysis, these authors reported that although chemical acidification resulted in an increased concentration, those doughs fermented with strains of *L. alimentarius*, *L. brevis*, *L. sanfranciscensis*, and *L. hilgardii* showed a greater concentration with some differences in the amino acid pattern. Further, two-dimensional electrophoresis revealed that both biological and chemical acidification caused a marked modification of the polypeptide pattern with respect to a nonacidified dough. The strain dependency of proteolysis was demonstrated by the fact that almost all of the polypeptides were hydrolyzed by the strains of *L. alimentarius*, *L. brevis*, and *L. sanfranciscensis* used, whereas the strain of *L. hilgardii* employed showed much less capacity for hydrolysis. The effect of the proteolytic activity of some of these strains on dough rheology measured using empirical techniques has also been shown to be more extensive than that seen for a chemically acidified equivalent (Di Cagno *et al*., 2002).

In addition to the proteolytic activity of lactic acid bacteria, the role of cereal proteases has been explored. Thiele *et al*. (2002) observed that the presence of lactobacilli had little effect on total amino acid concentrations in wheat sourdoughs when compared to acid aseptic doughs, thus concluding that the proteolytic activity of lactobacilli was negligible in comparison with that of wheat flour. The reduction in pH brought about by the lactic fermentation did, however, enhance proteolysis relative to neutral sterile doughs. These findings were confirmed in another study (Thiele *et al*., 2003) that used fluorescence labeling of wheat protein fractions to determine the degree of gluten hydrolysis and depolymerization during sourdough fermentation, which found that compared to the degradation of gliadin and glutenin proteins in aseptic acidified doughs, the additional proteolytic activity of microbial enzymes was small. This study did, however, report that microbial fermentation affected the size distribution of the peptides resulting from proteolytic degradation of wheat proteins in so far as the presence of lactobacilli promoted a decrease in the concentration of larger peptides and

an increase in that of smaller molecules such as dipeptides and amino acids. These authors concluded that proteolytic degradation of gluten proteins and depolymerization of the gluten macropolymer observed during sourdough fermentation were in the main attributable to dough pH and cereal enzyme activity.

The contribution made by cereal proteases to the structural changes seen during sourdough fermentation has been further explored via the use of a sterile nonacidified dough preferment (Clarke *et al*., 2004). In comparing the rheological properties of this dough with those of chemically acidified or biologically acidified dough preferments, this study showed that those changes associated with time were the main influence on the properties of all three preferments during a 24-hour fermentation period. There was a reduction in elasticity and firmness for all treatments during the fermentation period, at the end of which there was little difference between the rheological characteristics of the treatments, irrespective of the presence of acid. Relative to the neutral treatment, the biological and chemical acid treatments were more degraded at the end of fermentation, indicating that the structural changes were further enhanced by the presence of acid, in keeping with the descriptions of wheat flour proteolytic enzymes with acidic pH optima described earlier. Proteolytic activity attributable to wheat flour cereal proteases has also been reported by Kawamura and Yonezawa (1982). Using SDS-PAGE, these authors reported that the mode of action of these cereal proteases was relatively specific for a high-molecular-weight subunit of glutenin (90,000 Da) and that this subunit disappeared as a function of time while new protein bands in the region of 26,000–28,000 Da and a new protein band of 68,000 Da appeared. In the same vein, Bleukx *et al*. (1997) identified a range of proteolytic activities associated with vital wheat gluten, which also led to a disappearance of high- and low-molecular-weight glutenin subunits with the formation of new protein bands in the 30,000–33,000 Da region. Bleukx and Delcour (2000) identified a second aspartic proteinase associated with wheat gluten, which did not hydrolyze gluten when incubated with gluten alone. It was hypothesized, however, that such a proteinase might act synergistically with other proteases in gluten breakdown.

V. EFFECT OF SOURDOUGH INCORPORATION ON BREAD DOUGH STRUCTURE

Given that the rheology of wheat doughs and the resulting loaf volume are mainly determined by gluten proteins, any changes associated with proteolytic degradation during sourdough fermentation will no doubt have an

impact on the nature of the bread dough when prefermented material is incorporated. From a rheological point of view, it is well established that as fermentation progresses, there is a change in nature of the elements contributing to dough structure such as the decrease in the viscosity described for a gluten solution (Kawamura and Yonezawa, 1982). Using empirical techniques to measure the rheology of fermented doughs, Di Cagno *et al.* (2002) found a decrease in resistance to extension and an increase in both extensibility and degree of softening. Clarke *et al.* (2004) using fundamental techniques, reported that sourdough preferments became softer and less elastic as fermentation progressed. This study hypothesized that with time, large protein aggregates responsible for the dough's structural integrity are broken down into small protein aggregates by cereal proteases, resulting in a softer and less elastic system. The structural implications of these changes were examined using confocal laser-scanning microscopy, which revealed that during the course of fermentation, the gluten of a biologically acidified preferment underwent a transition from having a distinct structure in the form of strands to becoming more amorphous, a change consistent with the hypothesis that the protein is partially degraded during fermentation. It was inferred that a reduction in the quantity of polymeric proteins (i.e., glutenins) present in the preferment was effected by degradation, thus resulting in a less elastic system. It was apparent from the subsequent rheology data for the dough that the incorporation of 20% of the flour in the form of a preferment, be it sterile or acidified, reduced elasticity and firmness, thus yielding a significantly softer, less elastic dough than the control containing no added preferment. Confocal laser-scanning microscopy revealed that the effect of the incorporation of biologically acidified material could also be seen with regards to dough microstructure. Relative to the fine well-oriented network of the control, the gluten of the dough with added preferment had a more amorphous nature and there were greater areas of aggregated material composed of thicker proteinaceous strands in evidence. This scenario is resonant of that described by Kieffer and Stein (1999) for relaxed and reshaped wheat systems, where the presence of thicker strands could allow for a greater increase in loaf volume. From a technological point of view, it was hypothesized that the incorporation of an optimally degraded preferment may be the principal reason for the achievement of doughs that yield better loaf quality characteristics when sourdough is optimally applied.

In addition to the impact of sourdough on the structure and rheology of the constituent gluten proteins making up the framework of the dough, its effect on gas formation must also be considered because gas formation by microorganisms is necessary to obtain leavened bread. In the case of sourdough breads, carbon dioxide is produced by both lactic acid bacteria and yeast and the contribution of each group to the overall gas volume differs

with the type of starter culture and the dough technology applied (Hammes and Gänzle, 1998). For both types of microorganisms, the rate of production is dependent on a number of ecological factors including the nutrient supply, which depends in turn on the degradation of macromolecules such as protein and starch. The gas-holding capacity of the dough system, on the other hand depends on the physicochemical structure of the dough, which in the case of wheat dough, is mainly governed by the gluten network (Finney, 1943; Lookhart, 1997). In terms of evaluating the interactions between the two types of microorganisms using a rheofermentometer, Gobbetti *et al.* (1995) found that in comparison to that seen in sourdough produced with yeast alone, yeast fermentation was faster in the presence of heterofermentative lactic acid bacteria, whereas that with homofermentative bacteria was slower and produced more carbon dioxide. Hammes and Gänzle (1998) documented that for traditional (type I) sourdoughs, the contribution made by heterofermentative lactic acid bacteria to gas production is substantial and may even be decisive but that in the case of sourdoughs where baker's yeast is also applied during dough formation (type II sourdoughs), the quantity of gas produced by the sourdough microflora is only of minor importance. This effect was demonstrated in a report that used response surface methodology to examine the effects of sourdough fermentation time and yeast quantity on loaf quality parameters (Clarke *et al.*, 2003). An increase in loaf-specific volume associated with an increase in sourdough fermentation time was observed when no baker's yeast was applied. In the presence of yeast, however, the same trend was not in evidence, once more highlighting the overriding effect of gas production by yeasts relative to lactic acid bacteria. This same effect was also demonstrated by evaluation of the gaseous release characteristics of a range of doughs, all of which contained baker's yeast (Clarke *et al.*, 2002). This study found that there were no significant differences between the total amounts of carbon dioxide produced, lost, or retained by biologically acidified doughs relative to nonbiologically acidified dough as measured using a rheofermentometer. From a technological point of view, it may, therefore, be hypothesized that it is the gas retention, and not the gas production properties of the dough, that is improved when sourdough is applied.

A. INTERACTION BETWEEN SOURDOUGH AND DOUGH ADDITIVES

In addition to reliance on the integral components of dough, there is an increasing trend for the use of additives in the baking industry to achieve optimal functionality in terms of dough-handling properties and bread quality attributes, including shelf life (Rosell *et al.*, 2001). The interaction

between sourdough and a number of additives such as exogenous enzymes and nonstarch polysaccharides has been evaluated (Corsetti *et al*., 2000; Di Cagno *et al*., 2003). With respect to the rate of starch retrogradation during storage, Corsetti *et al*. (2000) used crumb firmness measures and differential scanning calorimetry experiments to determine the contribution made by the addition of α-amylase, protease, pentosans, and pentosanases to the rate of staling observed in sourdough breads prepared using strains of *S. cerevisiae*, *L. sanfranciscensis*, and *L. plantarum*. These authors reported that compared with a control bread, the positive effect seen for the sourdough bread was further enhanced by the addition of α-amylase. In breads where pentosans alone or a mixture of pentosans, endoxylanase, and a strain of *L. hilgardii* was added, an even greater delay in bread firming and staling was observed. These authors concluded that the use of a lactic acid bacterial strain with particular characteristics may be a fundamental prerequisite in the retardation of bread crumb firmness. A combined effect of sourdough lactic acid bacteria and pentosans was also proposed.

Di Cagno *et al*. (2003) studied the interactions between sourdough lactic acid bacteria and exogenous enzymes to optimize the effects on the microbial kinetics of acidification, acetic acid production, and textural properties of sourdough during the fermentation process. The enzymes used included glucose-oxidase, lipase, endoxylanase, α-amylase, or protease, enzymes that are typically applied to improve dough functionality. These authors found that of the 11 species of lactic acid bacteria used, only three were positively influenced by the addition of enzymes with regards to the rate and extent of lactic acidification. The use of enzymes in the context of sourdough may be difficult because the acidic environment may interfere with their activity. It was reported, however, that in some cases, the combined use of sourdough and enzymes could reduce the risk of dough weakening and the loss of gas-retention properties (Di Cagno *et al*., 2003).

B. USE OF LACTIC ACID BACTERIA METABOLITES TO REPLACE ADDITIVES

The production of exopolysaccharides by lactic acid bacteria during food fermentation is another interesting aspect of sourdough technology with the potential for the replacement of hydrocolloids. These compounds, commonly named *gums*, are used as texturizing, antistaling, or prebiotic additives in bread production (Tieking *et al*., 2003). Exopolysaccharides are microbial polysaccharides secreted extracellularly, the amount and structure of which depend on the particular microorganism present and the available carbon substrate (Korakli *et al*., 2001). Studies of the application of exopolysaccharide-forming starter cultures have focused primarily on

heteropolysaccharides from lactobacilli in dairy fermentations (Tieking *et al.*, 2003). The production from sucrose of a levan type of fructan in wheat doughs has, however, been described by Korakli *et al.* (2001). In view of the fact that the amount of exopolysaccharide produced corresponded to 0.5–1.0% on a flour weight basis, these authors concluded that the quantity would be sufficient to effect changes in the rheological properties of the dough, as well as in the textural and shelf-life parameters of the bread. In addition to the production of fructan, Tieking *et al.* (2003) also described the *in situ* production of glucan by sourdough bacterial strains in the context of wheat flour sourdough at levels ranging from 0.5 to 2 g/kg of flour. In view of the findings of Rosell *et al.* (2001) that the addition of 0.5% hydrocolloid had an impact on dough rheology and bread quality, Tieking *et al.* (2003) assumed that the amount of exopolysaccharide produced by lactic acid bacteria in the context of sourdough to be technologically relevant. That these substances can be metabolized by bifidobacteria is also of nutritional advantage.

VI. CONCLUSION

Preservation of foods by fermentation is a widely practiced and ancient technology. In view of their unique metabolic characteristics, lactic acid bacteria are involved in many fermentation processes including cereals and in particular sourdough. It is clear that the application of sourdough to wheat bread production does indeed present a complex set of circumstances for food scientists and technologists. There exist myriad microbial, technological, and processing dimensions that must be considered to produce cereal products of optimal quality. It is also clear that a great deal of research has been conducted in relation to the application of sourdough to baked goods, including a development of the understanding of the roles played by the principal endogenous and process parameters. Significant advances have been made in understanding the contributions made by the presence of acid, the fermentation period, and the role played by cereal proteases in terms of fundamental changes in dough rheology and quality characteristics. There has also been much progress in the development of tools that allow for the selection of key sourdough microorganisms for particular activities such as those concerned with enzymatic, antifungal, antimicrobial, nutritional, and additive replacement aspects. These developments are important from the point of view of both basic research and industrial applications. The application of sourdough confers many advantages on the quality of the baked goods produced. The prospect of increasing quality, shelf life, safety,

and the nutritional value of breads is of considerable economic impact and favorable from a consumer's perspective.

Given that there already exists a considerable body of knowledge regarding the role and activity of the microflora concerned with sourdough, the future challenges for research and development in the area must include the further improvement of reliability and product quality through optimization of starter culture performance and the elimination of those factors that impede the fermentation process. In this regard, it is to be anticipated that the considerable resources that have been devoted to the "biotechnology of lactic acid bacteria" over the past number of years will deliver results with respect to these objectives. There remains a need, however, for the continued use of an interdisciplinary approach that will ensure that biotechnological advances can indeed yield positive outcomes in terms of industrial applications and consumer satisfaction. This is particularly the case given that the prospect of metabolic engineering of strains of lactic acid bacteria to generate derivatives with new attributes is now a real one.

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