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Influence of Acidification on Dough Rheological Properties

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1. Introduction

Due to increasing consumer demands for more natural, tasty and healthy food, the traditional process of sourdough bread production has been enjoyed renewed success in recent years, (Brümmer & Lorenz, 1991; Thiele *et al.*, 2002; Lopez *et al.*, 2003). Sourdough was traditionally used as leavening agents until it was replaced by baker's yeast in the 19th century (Corsetti & Setanni, 2007). Today, sourdough is employed in the manufacture of a number of products, such as breads, cakes and crackers (De Vuyst & Gänzle, 2005). Some of these products have strictly regional and artisanal character; well some of these are widely distributed on the world market (De Vuyst & Neysens, 2005). Many wheat breads and cakes are characteristic for the Mediterranean countries, the San Francisco bay and Southern America, whereas a number of bakery preparations made with rye, wheat, barley, or mixtures of these flours are typical for Central Europe, Eastern Europe and Scandinavia (Stephan & Neumann, 1999).

In general, sourdough is mixture of flour and water that is than fermented with lactic acid bacteria (LAB) (Hammes & Gänzle, 1998). Ordinarily, these LAB are heterofermentative strains which produce lactic and acetic acid in the mixture, and resulting in a sour taste of the dough (Vogel *et al.*, 1999). The acidification process affected by the application of sourdoughs is mainly used to improve quality, taste and flavour of wheat breads (Brümmer & Lorenz, 1991; Katina *et al.*, 2006a; Arendt *et al.*, 2007), and the slow staling (Katina *et al.*, 2006b; Plessas *et al.*, 2007).

Sourdough can be freshly produced in bakeries, or can be obtained from the commercial suppliers in the some of the following applying forms: living, liquid sourdough or dried, non-fermenting sourdough (Böcker *et al.*, 1990). Considerably, doughs could be acidified biologically, chemically and naturally preferment.

The addition of sourdough during production of wheat bread causes major changes in the dough characteristics (Clarke *et al.*, 2002; Clarke *et al.*, 2004; Ketabi *et al.*, 2008), especially in the flavour and structure (Clarke *et al.*, 2002). The effect of the fermentation process of wheat doughs containing lactic acid bacteria are complex and depending on variations between sourdoughs regard to the type of starter culture, dough yield and fermentation regime used (Wehrle *et al.*, 1997). This propounds requirements for the detail microbiological investigations on sourdough microflora. Study of sourdough from

microbiological point of view barely started a hundred years ago (Salovaara, 1998). Today, microbial population of different types of sourdough is rather known. Many inherent properties of sourdough rely on the metabolic activities of its resident LAB: lactic fermentation, acetic fermentation, proteolysis, synthesis of volatile compounds, anti-mould activity and antiropiness are among the most important activities during sourdough fermentation (Hammess and Gänzle, 1998). The most of the effects of sourdough have been considered by pH value decrease which has been caused with organic acid production.

Between many of important effects, the drop in pH value caused by the organic acids produced influences the viscoelastic behaviour of doughs, respectively to sourdoughs rheological properties (Wehrle & Arendt, 1998). A correct description of the changes in dough behaviour is necessary to maintain handling and machinability in industrial production (Wehrle et al., 1997). Furthermore, following the identification and classification of LAB from cereal fermentation, basic and applied today face the challenge of identifying functional characters of these bacteria to completely exploit their microbial metabolic potential from the production of baked goods (Vogel et al., 2002). In European countries, production of sourdoughs from wheat flour has been predominant. Most fundamental studies on wheat sourdough have been conducted in Western Europe, and the results have been published in German-language journals and books (Brümmer & Lorenz, 1991).

Rheological properties, acidification and flavour development are the most important parameters in fermentation process control (Hames et al., 2005). Rheological properties of dough have been determined by a number of methods, such as dynamic rheological measurements, extensigraphs, alveographs, lubricated uniaxial compression, oscillatory probe rheometers etc. (Hoseney, 1994). The rheological characteristics of dough have been considerably changed with fermentation. Types of microorganisms, metabolic activity and time-dependent development pH value effect on rheological properties (Wehrle & Arendt, 1998). Acids strongly influence on the mixing properties of dough. Doughs with lower pH requires a slightly shorter mixing time and have less stability than dough with normal pH level (Hoseney, 1994). Changes in pH values caused by production of lactic acid also influence on the rheological properties of dough (Wehrle et al., 1997). Dough with containing acid has been characterized by increased phase angle and reduced complex modulus indicative of overmixing (Wehrle et al 1997). Small physical and chemical changes in the gluten network can result in significant changes in rheological properties. Clarke et al. (2002) were concluded that addition of sourdough prepared either from a single strain starter culture or a mixed strain starter culture had significant impact on the rheological properties of wheat flour dough.

Koceva Komlenić et al (2010) investigated the influence of chemical and biological acidification on dough rheological properties. According to their experimental results, rheological properties strongly depend on acidification type. Dough with lower pH value showed less stability during mixing, decreased extensibility and gelatinization maximum. In general, the rheological properties of dough greatly improved when the sourdough was added.

Regard to all facts mentioned above, fermentation of dough with LAB greatly effects on the properties of many bakery products. Structure properties are one of the most important. From that reason, rheological measurements play important role in definition of quality of products from sourdough. In this paper, influence of sourdough fermentation on the structure of sourdough was observed.

2. Microbiology of sourdough

It is well known that the type of bacterial flora developed in each fermented food depends on water activity, pH (acidity), minerals concentration, gas concentration, incubation temperature and composition of food matrix (Font de Valdez et. al. 2010). The microflora of raw cereals is composed of bacteria, yeast and fungi ($10^4 - 10^7$ CFU/g), while flour usually contains $2 \times 10^4 - 6 \times 10^6$ CFU/g (Stolz, 1999). In sourdough fermentation major role play heterofermentative species of LAB (Salovaara, 1998; Corsetti & Settani, 2007), especially when sourdoughs are prepared in a traditional manner (Corsetti et. al., 2003).

Type Ia	Type Ib	Type Ic	Type II	Type III
Obligate heterofermentative	Obligate heterofermentative	Obligate heterofermentative	Obligate heterofermentative	Obligate heterofermentative
<i>Lb. sanfranciscensis</i>	<i>Lb. brevis</i> <i>Lb. brevis</i> <i>Lb. buchneri</i> <i>Lb. fermentum</i> <i>Lb. fructivorans</i> <i>Lb. pontis</i> <i>Lb. reuteri</i> <i>Lb. sanfranciscensis</i> <i>W. cibara</i>	<i>Lb. pontis</i> <i>Lb. fermentum</i> <i>Lb. reuteri</i>	<i>Lb. brevis</i> <i>Lb. fermentum</i> <i>Lb. frumenti</i> <i>Lb. pontis</i> <i>Lb. panis</i> <i>Lb. reuteri</i> <i>Lb. sanfranciscensis</i> <i>W. confusa</i>	<i>Lb. brevis</i>
	Facultative heterofermentative <i>Lb. alimentarius</i> <i>Lb. casei</i> <i>Lb. paralimentarius</i> <i>Lb. plantarum</i>			Facultative heterofermentative <i>Lb. plantarum</i> <i>P. pentosaceus</i>
	Obligate homofermentative <i>Lb. acidophilus</i> <i>Lb. delbrueckii</i> <i>Lb. farciminis</i> <i>Lb. mindensis</i>	Obligate homofermentative <i>Lb. amylovorus</i>	Obligate homofermentative <i>Lb. acidophilus</i> <i>Lb. delbrueckii</i> <i>Lb. amylovorus</i> (rye) <i>Lb. farciminis</i> <i>Lb. johnsonii</i>	

Table 1. Classification of sourdoughs and the corresponding characteristic microflora (De Vuyst & Neysens, 2005)

Sourdough LAB generally belongs to the genus *Lactobacillus*, *Leuconostoc*, *Pediococcus* or *Weisella* (Ehrmann & Vogel, 2005; De Vuyst & Neysens, 2005). A few less than 50 different species of LAB from sourdough have been reported by De Vuyst & Neysens (2005). Although species belonging *Leuconostoc*, *Pediococcus*, *Weisella*, *Enterococcus* and *Streptococcus* genera have been isolated from sourdough, *Lactobacillus* strains are the most frequently observed bacteria in sourdoughs (Corsetti & Settanni, 2007). Characteristic microflora of sourdough is presented in Table 1. Undesirable bacteria such as *Staphylococcus aureus* and *Bacillus cereus*, as well as other bacteria, may be present in minor concentration (De Vuyst and Neysens, 2005). Corsetti & Settani (2007) reported that the recently described species *Lactobacillus frumenti*, *Lactobacillus mindensis*, *Lactobacillus paralimentarius*, *Lactobacillus*

spicheri, *Lactobacillus rossiae*, *Lactobacillus acidifarinae*, *Lactobacillus zymae*, *Lactobacillus hammesii*, *Lactobacillus nantensis* and *Lactobacillus siliginis* were first isolated from the sourdough matrices. 15 lactobacilli species known to occur in sourdough are also known to live in human and animal intestines. *Lactobacillus reuteri*, *Lactobacillus acidophilus* and *Lactobacillus plantarum* are some of these intestinal lactobacilli (Hammes & Gänzle, 1998).

Kumar et. al. (2004) reported a phylogram based on 16S rRNA gene sequences of sourdough lactobacilli. A total of 41 species are included in phylogram tree, some of them, such as *Lactobacillus rhamnosus*, have been rarely or only once reported to be found in sourdough (Spicher & Löner, 1985). Hammes et. al. (2005) reported that about 30 species are considered to be typical of sourdough environments. *Lactobacillus sanfranciscensis*, *Lactobacillus brevis* and *Lactobacillus plantarum* are the most frequently lactobacilli isolated from sourdough (Gobbetti, 1998; Corsetti et. al. 2001; Valmorri et. al., 2006; Corsetti & Settanni, 2007). *Lb. sanfranciscensis* was first reported in the San Francisco sourdough French bread process, to be responsible for acid production (Corsetti et. al., 2001). *Lb. sanfranciscensis* has been predominant bacteria in traditional production by various stages of continuous production and production by commercial starter cultures (Gobbetti & Corsetti, 1997). Gobbetti et. al. (1998) reported on the *Lb. sanfranciscensis* – *Lb. plantarum* association in Italian wheat sourdough. *Lb. alimentarius*, which probably belong to *Lb. paralimentarius*, were first isolated from Japanese sourdough (Cai et. al., 1999). *Lb. brevis* and *Lb. plantarum* have been associated with *Lb. fermentum* in Russian sourdoughs (Kazayanska et. al., 1983). *Lb. fermentum* dominates Swedish sourdoughs and German type II sourdoughs (Meroth et al., 2004.) Gobbetti et. al. (1994b) reported that *Lb. acidophilus* is found in Umbrian (Italian region), while Corsetti et. al. (2003) described a new sourdough associated species, *Lb. rossae*, in sourdoughs of central and southern Italy (Settanni et. al., 2005a). African sorghum sourdoughs, which are produced at higher temperature (> 35 °C) contain obligate heterofermentative *Lb. fermentum*, *Lb. pontis* and *Lb. reuteri* species, as well as the obligate homofermentative *Lb. amylovorus* (De Vuyst & Neysens, 2005).

According to all facts mentioned above, it is so clear that large biodiversity in lactobacilli composition of the sourdough exists, regarding to type of sourdough (Table 2), as well as to some cultural, geographical and traditional reasons.

Country	Product/method of isolation and identification	Lactic acid bacteria
Belgium	Wheat/rye sourdoughs polyphasic approach	<i>Lb. brevis</i> , <i>Lb. plantarum</i> , <i>Lb. sanfranciscensis</i> , <i>Lb. paralimentarius</i> , <i>P. pentosaceus</i> , <i>Lb. helveticus</i>
Finland	Rye sourdough phenotypical	<i>Lb. acidophilus</i> , <i>Lb. plantarum</i> , <i>Lb. casei</i>
Denmark	Sour rye dough phenotypical	<i>Lb. reuteri</i> , <i>Lb. panis</i> , <i>Lb. amylovorus</i>
France	Wheat bread phenotypical	<i>Lb. plantarum</i> , <i>Lb. casei</i> , <i>Lb. delbrueckii</i> subsp. <i>delbrueckii</i> , <i>Lb. acidophilus</i> , <i>Lb. brevis</i> , <i>Leuc. mesenteroides</i> subsp. <i>mesenteroides</i> , <i>Leuc. mesenteroides</i> subsp. <i>dextranicum</i> , <i>P. pentosaceus</i> , <i>Lb. Curvatus</i>
Germany	Wheat sourdough phenotypical	<i>Lb. delbrueckii</i> , <i>Lb. plantarum</i> , <i>Lb. casei</i> , <i>Lb. fermentum</i> , <i>Lb. buchneri</i> , <i>Lb. brevis</i>
	Rye bread phenotypical	<i>Lb. acidophilus</i> , <i>Lb. farciminis</i> , <i>Lb. alimentarius</i> , <i>Lb. casei</i> , <i>Lb. plantarum</i> , <i>Lb. brevis</i> , <i>Lb. sanfranciscensis</i> , <i>Lb. fructivorans</i> , <i>Lb. fermentum</i> , <i>Lb. buchneri</i>

Country	Product/method of isolation and identification	Lactic acid bacteria
	Rye sourdough phenotypical	<i>Lb. acidophilus</i> , <i>Lb. casei</i> , <i>Lb. plantarum</i> , <i>Lb. farciminis</i> , <i>Lb. alimentarius</i> , <i>Lb. brevis</i> , <i>Lb. buchneri</i> , <i>Lb. fermentum</i> , <i>Lb. fructivorans</i> , <i>Lb. sanfranciscensis</i> , <i>Pediococcus</i> spp.
	Wheat sourdoughs (Panettone, wheat bread) phenotypical	<i>Lb. plantarum</i> , <i>Lb. casei</i> , <i>Lb. farciminis</i> , <i>Lb. homohiochii</i> , <i>Lb. brevis</i> , <i>Lb. hilgardii</i> (spontaneous); <i>Lb. sanfranciscensis</i> , <i>Lb. brevis</i> , <i>Lb. hilgardii</i> , <i>W. viridescens</i> (masa madre)
	Rye sourdough RAPD-PCR	<i>Lb. amylovorus</i> , <i>Lb. pontis</i> , <i>Lb. frumenti</i> , <i>Lb. reuteri</i>
	Rye bran PCR-DGGE	<i>Lb. sanfranciscensis</i> , <i>Lb. mindensis</i> (type I rye sourdough); <i>Lb. crispatus</i> , <i>Lb. pontis</i> , <i>Lb. panis</i> , <i>Lb. fermentum</i> , <i>Lb. frumenti</i> (type II rye sourdough); <i>Lb. johnsonii</i> , <i>Lb. reuteri</i> (type II rye bran sourdough)
Greece	Wheat sourdoughs polyphasic approach	<i>Lb. sanfranciscensis</i> , <i>Lb. brevis</i> , <i>Lactobacillus</i> spp. ^a , <i>Lb. paralimentarius</i> , <i>W. cibaria</i>
Italy	Panettone phenotypical	<i>Lb. brevis</i> , <i>Lb. plantarum</i>
	Panettone, Brioche phenotypical	<i>Lb. sanfranciscensis</i> , <i>Lb. fermentum</i> , <i>Lb. plantarum</i> , <i>Leuc. mesenteroides</i> , <i>Pediococcus</i> spp.
	Umbrian wheat sourdoughs phenotypical	<i>Lb. sanfranciscensis</i> , <i>Lb. plantarum</i> , <i>Lb. farciminis</i>
	Pizza (Naples) phenotypical	<i>Lb. sakei</i> , <i>Lb. plantarum</i> , <i>Leuc. gelidum</i> , <i>Leuc. mesenteroides</i>
	Verona sourdoughs RAPD-PCR	<i>Lb. sanfranciscensis</i>
	Lombardian mother sponges	<i>Lb. sanfranciscensis</i>
	speciesspecific PCR	
	Apulian wheat sourdoughs 16S rDNA sequencing 16S/23S rRNA spacer region PCR	<i>Lb. sanfranciscensis</i> , <i>Lb. alimentarius</i> , <i>Lb. brevis</i> , <i>Leuc. citreum</i> , <i>Lb. plantarum</i> , <i>Lb. lactis</i> subsp. <i>lactis</i> , <i>Lb. fennentum</i> , <i>Lb. acidophilus</i> , <i>W. confusa</i> , <i>Lb. delbmeckii</i> subsp. <i>bulgaricus</i>
Iran	Sangak phenotypical	<i>Leuc. mesenteroides</i> , <i>Lb. plantarum</i> , <i>Lb. brevis</i> , <i>P. cerevisiae</i>
Mexico	Pozol (maize) 16S rDNA sequencing	<i>Lb. lactis</i> , <i>S. suis</i> , <i>Lb. plantarum</i> , <i>Lb. casei</i> , <i>Lb. alimentarius</i> , <i>Lb. delbrueckii</i>
Morocco	Sourdough ferments traditional starter sponges phenotypical	<i>Lb. plantarum</i> , <i>Lb. brevis</i> , <i>Lb. buchneri</i> , <i>Lb. casei</i> , <i>Leuc. mesenteroides</i> , <i>Pediococcus</i> sp.
	Soft wheat flour phenotypical	<i>Lb. plantarum</i> , <i>Lb. delbrueckii</i> , <i>Lb. buchneri</i> , <i>Lb. casei</i> , <i>Lb. sanfranciscensis</i> , <i>Leuc. mesenteroides</i> , <i>P. pentosaceus</i>
Portugal	Broa phenotypical	<i>Leuconostoc</i> spp., <i>Lb. brevis</i> , <i>Lb. curvatus</i> , <i>Lb. delbrueckii</i> , <i>Lb. lactis</i> subsp. <i>lactis</i> , <i>E. casseliflavus</i> , <i>E. durans</i> , <i>E. faecium</i> , <i>S. constellatus</i> , <i>S. equinus</i>
Russia	Rye sourdough phenotypical	<i>Lb. plantarum</i> , <i>Lb. brevis</i> , <i>Lb. fermentum</i>
Spain	Wheat sourdough phenotypical	<i>Lb. brevis</i> , <i>Lb. plantarum</i>
	Wheat sourdough phenotypical	<i>Lb. brevis</i> , <i>Lb. plantarum</i> , <i>Lb. cellobiosus</i> , <i>Leuc. mesenteroides</i>
Sudan	Kisra (sorghum sourdough)	<i>Lb. fermentum</i> , <i>Lb. reuteri</i> , <i>Lb. amylovorus</i>
	Kisra RAPD	<i>E. faecalis</i> , <i>Lb. laclis</i> , <i>Lb. fermentum</i> , <i>Lb. reuteri</i> , <i>Lb. vaginalis</i> , <i>Lb. helveticus</i>
Sweden	Rye/wheat phenotypical	<i>Lb. fermentum</i> , <i>Lb. delbrueckii</i> , <i>Lb. acidophilus</i> , <i>Lb. plantarum</i> , <i>Lb. rhamnosus</i> , <i>Lb. farciminis</i> , <i>Lb. fermentum</i> , <i>Lb. sanfranciscensis</i> , <i>Lb. brevis</i> , <i>W. viridescens</i>

Country	Product/method of isolation and identification	Lactic acid bacteria
USA	Rye sourdough phenotypical	<i>Lactobacillus</i> sp., <i>P. pentosaceus</i>
	San Francisco sourdough French bread phenotypical	<i>Lb. sanfranciscensis</i>

Table 2. Biodiversity of sourdough lactic acid bacteria in sourdough of different origin (De Vuyst & Neysens, 2005)

The following yeasts have been detected in cereals (9×10^4 CFU/g) and flour (2×10^3 CFU/g): *Candida*, *Cryptococcus*, *Pichia*, *Rodothorula*, *Torulaspora*, *Trychoporon*, *Saccharomyces* and *Sporobolomyces*. *Saccharomyces cerevisiae* is not found in the raw materials. Its occurrence in sourdough has been explained by the application of baker's yeast in most daily bakery practice (Corsetti et. al., 2001). Among fungi (ca. 3×10^4), *Alternaria*, *Cladosporium*, *Drechslera*, *Fusarium*; *Helminthosporium*, *Ulocladium*; *Aspergillus* and *Penicillium* were found in raw cereals and flour. Possibility to dough be contaminated with these species exist, but in the most of cases it could be avoided (De Vuyst & Neysens, 2005).

3. Role of LAB in sourdough fermentation

The metabolic activities of lactobacilli during sourdough fermentation improve dough properties, bread texture and flavour; retard the stalling process of bread; and prevent bread from mould and bacterial spoilage (Gerez et. al., 2009; de Valdez et. al., 2010). Additionally, LAB could contribute to nutritive value and healthiness of bread (Gobbetti et. a., 2007). The sourdough microflora is usually composed of stable associations of yeasts and lactobacilli.

3.1 Carbohydrate metabolism

In dough fermentation carbohydrate metabolism varies depending on involved LAB strains, type of sugars, presence of yeasts and processing conditions (Gobetti et. al., 1994a). The importance of antagonistic and synergistic interactions between lactobacilli and yeasts are based on the metabolism of carbon hydrates and amino acids and the production of carbon dioxide (Gobetti & Corsetti 1997). Lactic and acetic acid are predominant products of sourdough fermentation (Figure 1).

Diversity in metabolic pathways between different sourdoughs is large. Recipe and geographical origin make the difference in the aroma of breads consumed in the different areas (Hansen & Schieberle, 2005). All these parameters influence on characteristics and give originality to different breads from sourdoughs. It is the basic reason why bread from biologically fermented dough posses a superior sensory quality than bread produced from chemically acidified dough (Kirchhoff & Schieberle, 2002). The ratio between lactic and acetic acid is an important factor that might affect the aroma profile and structure of final product. Acetic acid, produced by heterofermentative LAB, is responsible for a shorter and harder gluten, while lactic acid can gradually account for a more elastic gluten structure (Lorenz, 1983; Corsetti & Settani, 2007). *Lb. sanfranciensis*, isolated from traditional Italian sourdoughs ferment only glucose and maltose. However, *Lb. sanfranciensis* strains isolated from some other sourdoughs use other sugars, such as sucrose, raffinose, galactose, melobiose, ribose and fructose (Tieking et al., 2003). Ginés et. al. (1997) presented metabolic potential of *Lb. reuteri* CRL1100, a strain isolated from homemade sourdough. This

microorganism metabolizes glucose and galactose, but not fructose and cellobiose. The most of lactobacilli strain in sourdough are unable to ferment sucrose, a disaccharide whose metabolism by heterofermentative lactobacilli in sourdough appears to be attributable to glycosyl-transferases rather than to invertase activities (Font de Valdez et al., 2010). Recently, it has been reported that certain lactic acid bacteria are able to produce exopolysaccharides (EPS), which might have a positive affect on bread volume and shelf life (Font de Valdez et al., 2010). Following EPS have been produced during fermentation of sourdough: fructans (levan, inulin) and glucans (reuteran, dextran, xanthan). EPS influence significantly to the texture improvement of sourdough and bread (Tieking et al., 2003). The production of EPS in sufficient amounts during sourdough fermentation would create the possibility to replace hydrocolloids in baking (Tieking et al., 2003). Hydrocolloids have been reported to improve bread quality through stabilization of water-flour association in sourdoughs matrix (Tieking et al., 2003; Font de Valdez et al., 2010).

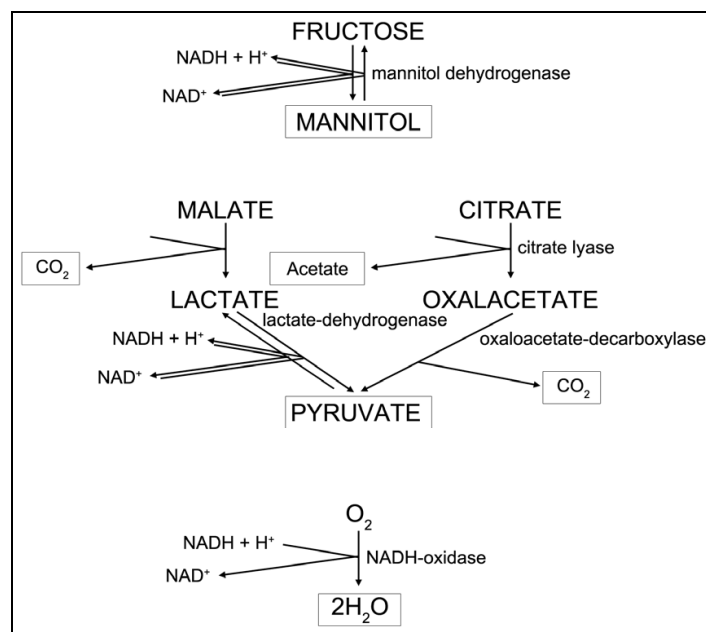


Fig. 1. Fate of potential electron acceptors upon carbohydrates fermentation by *Lb. sanfranciscensis* (Corsetti et al., 2007)

3.2 Metabolism of proteins

During sourdough fermentations protein modification and protein degradation have been occurred. Protein degradation that occurs during sourdough fermentations is among the key metabolic route that directly effects on dough texture and flavour of sourdough. During dough fermentation, LAB releases small peptides and free amino acids important for microbial growth and acidification of dough (Rollán & Font de Valdez, 2001). Also, small peptides and free amino acids are important as precursors for flavour development of the leavened baked products (Thiele et al., 2002). According to the results of studies performed by Gerez et al. (2006) 13 nine lactobacilli and four pediococci were able to use gluten as a nitrogen source. Gerez et al. (2006) also reported an increase in essential amino acids (treonine, valine, lysine and phenylalanine) in a gluten based medium fermented by LAB strains. Thiele et al. (2004) reported that the degree of protein degradation in wheat

sourdough is usually similar to that observed in chemically acidified dough. However, bacterial proteolysis during sourdough fermentation was shown to contribute much more to the development of typical sourdough flavours of baked breads compared to breads produced from acidified or yeasted dough (Hansen et. al., 1989a, Hansen et. al., 1989b). Gluten proteins determine, to a great extent, the rheological properties of wheat dough. Substantial hydrolysis of gliadin and glutenin proteins occurs during sourdough fermentation. Proteolytic activity in sourdough originates not only from LAB enzymes, but also derives from the cereal materials present in sourdough (Thiele, 2002; Thiele, 2004). Except activity of own enzymes, LAB contribute to overall proteolysis during sourdough fermentation by creating optimum (acidic) conditions for activity of cereal proteinases (Vermeulen et al. 2006). The partial hydrolysis of glutenins during sourdough fermentation results in depolymerisation and solubilisation of the gluten macro peptide (GMP). After 24 hours of fermentation with defined lactobacilli strains, all gluten proteins were SDS-soluble (Thiele et. al., 2003). Glutathione (GSH) is the most relevant reducing agent in wheat doughs (Grosh & Wieser, 1999). Heterofermentative lactobacilli express glutathione reductase during growth in dough and reduce extracellular oxidized glutathione (GSSG) (Jänsch et. al., 2007). The continuous transformation of GSSG to GSH by LAB metabolism maintains high SH levels in wheat doughs, and increase the amount of SH-groups in gluten proteins (Vermeulen et. al., 2006)

Increased proteolysis during sourdough fermentation leads to the liberation of amino acids in wheat dough. Importance of amino acid conversion to typical flavour volatile compounds has been reported by a number of researchers (Juillard et. al., 1998; Tamman et. al., 2000; Zotta et al. 2006). Sourdough fermentation with LAB results in an increase of amino acids concentration during fermentation, whereas dough fermentation with yeast reduces the concentration of free amino acids (Thiele et. al., 2002). Furthermore Gassenmayer & Schieberle (1995) reported that addition of amino acids (e.g. ornithine, leucine and phenylalanine) to dough resulted in an enhanced conversion to flavour compounds. The level of individual amino acids in wheat dough depends on the pH level of dough, fermentation time and the consumption of amino acids by the fermentative microflora (Thiele et. al., 2002). In wheat sourdoughs, *Lb. brevis linderi*, *Lb. safransciensis*, *Lb. brevis* and *Lb. plantarum* have been reported to increase the levels of aliphatic, dicarboxylic and hydroxyl amino acids (Gobbetti et. al., 1994a, Gobbetti et. al., 1994b). The yeasts, *S. cerevisiae* and *S. exiguus* decrease the total level of amino acids.

3.3 Development of aroma compounds

The key degradation reaction of amino acids during fermentation of sourdough is the Erlich pathway, leading to aldehydes or the corresponding alcohol, while during baking takes place the Strecker reaction which also lead to aldehydes, but also to the corresponding acids (Hoffmann & Schieberle, 2000). Differences other than acetic acid production, in the overall aroma profile of final bread depend to the type of dominating lactobacilli, as well as to the type of flour. E. g., firm wheat fermented with heterofermentative strains had higher content of ethyl acetate and hexyl acetate compared to cases when the homofermentative strains were used (Lund et. al., 1989). On the other hand, higher content of aldehydes was higher in rye sourdough fermented with homofermentative LAB (Lund et. al., 1989).

The arginine metabolism by *Lb. pontis* and *Lb. sanfransciensis* has been demonstrated to have an impact on bread flavour (Thiele et. al., 2002; De Angelis et. al., 2002). Phenylalanine metabolism was studied in *Lb. plantarum* and *Lb. sanfransciensis*, besides producing phenyllactic acid and 4-hydroxy compounds, which have documented antifungal activity (Vermeulen et. al., 2006). Gerez et. al. (2006) demonstrated that gluten-breakdown with lactobacilli and pediococci, beside reducing gluten-allergen compounds. Furthermore increased the basic amino acid concentration in broth cultures, mainly due to an increase in the ornithine amount, which is considered to be key flavour precursor in wheat bread, generating 2-acetyl-pyrolin (Gassenmeyer & Schieberle, 1995; Thiele et. a., 2002). Czerny & Schieberle (2002) reported that during fermentation of dough, LAB did not generate new aroma compounds than those present in raw materials. However, they demonstrated that many compounds as acetic acid and 3-methylbutanal were increased, whereas aldehydes were decreased.

From all referred above, LAB play very important role in overall sourdough fermentation process. Together with yeasts is responsible for unique quality of the baking bread. More research, however, is needed to clarify all metabolic pathways during fermentation of sourdough.

4. Classification of sourdoughs

Sourdoughs have been classified on the base of procedures during their production (Böcker et. al., 1995). Sourdoughs have been classified into three types:

1. type I sourdoughs or traditional sourdoughs
2. type II sourdoughs or accelerated sourdoughs
3. type III sourdoughs or dried sourdoughs

Each type of sourdough is characterised by a specific sourdough LAB microflora (Table 1).

4.1 Type I sourdough

Type I sourdoughs are produced with traditional techniques, and are characterized by continuous (daily) refreshments to keep the microorganisms in an active state. Type I sourdough is indicated by high metabolic activity, above all regard to leavening, i. e. gas production. The process is conducted at room temperature (20 - 30 °C) and the pH is approximately 4.0. Examples of baked breads with type I sourdough are Francisco sourdough French bread, Panettone and other brioches, Toscanon and Altamura bread, Pugliese, and three-stage sourdough rye bread. Traditional, type I sourdough encompass pure culture, pasty sourdough starter preparations from different origin (type Ia), spontaneously developed, mixed culture sourdoughs made from wheat and rye or mixture thereof and prepared through multiple stage fermentation processes (type Ib), and sourdough made in tropical regions fermented at high temperatures (Stolz, 1999). Pure culture sourdoughs (type Ia) are derived from natural sourdough fermentations.

4.2 Type II sourdough

Type II of sourdough has been prepared in semi-fluid silo condition. Those bakery pre-products serve mainly as dough acidifiers. Several modified, accelerated processes with

continuous propagation and long-term one-step fermentations are common now. They guarantee more production reliability and flexibility. A recent trend of industrial bakeries exists in the instalment of continuous sourdough fermentation plants (Stolz & Böcker, 1996).

Typical type II process last for 2-5 days and are often carried out at increased fermentation temperature (usually $> 30\text{ }^{\circ}\text{C}$) to speed up the process. Those sourdoughs exhibit a high acid content at a pH of < 3.5 after 24 hours of fermentation. The microorganisms are commonly in the late stationary phase and therefore exhibit restricted metabolic activity. The high dough yields of these preparations permit pumping of the dough. They are frequently used in local bakeries. Those sourdoughs are stored fresh until use (up to one week), they can be produced in large quantities. In industry, they are applied for the production of dried sourdough products as well (De Vuyst & Neysens, 2005).

4.3 Type III sourdough

Type III sourdoughs are dried doughs in powder form, which are initiated by defined starter cultures. They are used as acidifier supplements and aroma carries during bread-making. They mostly contain LAB that are resistant to drying and are able to survive in that form, e.g. heterofermentative *Lb. brevis*, facultative heterofermentative *P. pentosaceus* and *Lb. plantarum* strains. The drying process (spray-drying or drum-drying) also leads to an increased shelf-life of the sourdough and turns it into a stock product until further use. Dried sourdoughs are convenient, simple in use, and result in standardized end products. They can be distinguished in colour, aroma and acid content (De Vuyst & Neysens, 2005).

5. Rheology of the sourdough: Influence of LAB action

5.1 Effects of LAB to dough structure

Cereal grains contain starch and non-starch polysaccharides, the latter composed of glucose (β -glucans), fructose (polyfructan), xylose and arabinose (arabinoxylan) (Belitz & Grosh, 1999). Starch is partially digestible, while some other polysaccharides are not, and would represent dietary fibres. Wheat flours contain, in addition to polyfructans, also nystose, kestose and other fructooligosaccharides of the inulin type (Campbell et al., 1999). Their prebiotic potential has been well examined and documented (Van Loo et al., 1999; Corsetti & Settani, 2007). The structural effects of sourdough in wheat-based system may first be due to the direct influence of low pH on structure-forming dough components, such as gluten, starch, arabinoxylan etc. (Angioloni et al., 2006). Dough is very sensitive to changes in ionic strength and pH and such changes could have direct impact on the constituents of dough (Clarke et al., 2002). The drop in pH value caused by the produced organic acids influences the viscoelastic behaviour of dough. A correct description of the changes in dough behaviour is necessary to maintain handling and machinability in industrialized production (Wehrle et al., 1997). A number of earlier studies have examined influence of acids and different pH values on the dough properties. All of these confirmed that changes in the absolute pH value of sourdough significantly influence sourdough components. It has been well documented (Tsen, 1966; Wehrle et al., 1997; Takeda et al., 2001; Tieking et al., 2003) and supported by the findings of the many rheological studies which indicated differences between the nonacidified and chemically or biologically acidified doughs (Clarke et al., 2002). Osborne (1907) reported a century ago that the presence of acids increased the

solubility of the glutenin fraction extracted from the wheat flour. Barber et. al. (1992) reported that there could be mild acid hydrolysis on starch in sourdough system.

The pH profile may affect the time frame during which the acid influences the constituent ingredients of the dough. The changing pH values during sourdough fermentation period may also afford passage through a range of pH values close to the optimum for various enzymes present in the dough system. It is so-called secondary (indirect) effect of sourdough acidification (Clarke et al., 2004). The activity of proteolytic and amyolytic enzyme present may be influenced to a greater degree by the pH profile of the biological acidification fermentation period in contrast to the rather instantaneous nature of the chemically acidified regime. Optimum activity of these enzymes, which play significant role in changes of dough constituents, achieve optimum activity at pH 4-5 for the proteolytic and pH 3.6 - 6.2 for the amyolytic enzymes (Belitz & Grosh, 1992). Other enzymes that might affect the structural components of the dough the activity of which is pH dependent include peroxidases, catalases, lipoxigenases and polyphenol oxydases (Belitz & Grosh, 1992; Clarke et. al., 2002). Results obtained by the the fundamental rheological tests, baking tests, and farinograms show that activity of some enzymes in the biologically acidified dough led to structural changes in the dough (Corsetti et. al., 2000; Clarke et. al., 2002; Clarke et. al., 2004). Corsetti et. al. (2000) also reported that even limited photolytic degradation of wheat proteins affects the physical properties of gluten, which in turn can have a major effect on bread firmness and staling.

Gas production during fermentation of sourdough has been marked as a one of the most important parameters to affect on sourdough structure and rheology. Hammes and Gänzle (1998) have noted that the contribution of yeasts and LAB to the overall gas volume differs with the type of starters and the dough technology applied. These authors have also reported that gas formation by the sourdough microflora is only of minor importance if baker's yeast added. Clarke et. al. (2002) proved that the amount of gas produced by the sourdough microorganisms does not contribute remarkably to the increase in loaf specific volume. However, Hammes & Vogel (1995) indicated certain differences connected to the type of used LAB starter. Thus, obligately heterofermentative *Lb. pontis* significantly higher influenced to the gas formation in sourdough than facultatively hetrofermentative *Lb. plantarum*.

Exopolysaccharides (EPS) in sourdough are products of LAB metabolism. EPS have been recognized as the one of the important structure stability factors (Tieking et. al., 2003). Two classes of EPS from LAB can be distinguished, extracellular synthesized homopolysaccharides (HoPS), composed of only one type of monosaccharide and are synthesised by extracellular glucan and fructosyltransferases (glycosyltransferases) using sucrose as the glycosyl donor, and heteropolysaccharides (HePS) with irregular repeating units (Corsetti & Settani, 2007). HoPS are today generally applied to improve the texture of baked goods, while HePS usually have been used only in fermented dairy industry (Laws & Marshall, 2001). Sourdough lactobacilli have not been found to produce HePS.

One of the key sourdough LAB starters, *Lb. sanfranciscensis*, has been well known and characterized for its contribution to the enhancement of polysaccharide content due to the production of EPS (Korakli et. al., 2001; Korakli et. al., 2003; Tieking et. al., 2003). Formation of EPS is a well accepted characteristic of sourdough LAB, since this feature influencing on the viscosity of sourdough (Vogel et. al., 2002).

Such as presented, through the many microbiological and biochemical reaction starter microorganisms in sourdough affect on its important structural changes. From that reason, choose of adequate starters and control of structural changes during process of fermentation and baking is essential.

5.2 Dough structure

The main constituents are those derived from the flour, most importantly the proteins, both soluble and insoluble. The carbohydrates, which include starch, sugars, soluble and insoluble polysaccharides, are the most abundant. The lipids form a small, but significant part of the flour. The molecules of some minor constituents are composed of the lipid part (glycolipids) or are protein part (lipoproteins). These molecules are interesting because of their possible role in the interaction between more abundant constituents. Finally, flour enzymes may affect the dough properties (Chavan & Chavan, 2011). Water, one of the basic constituents, plays a key role in the formation of dough. During dough mixing, some air is occluded. The air forms the nuclei of the gas cells, which expand during fermentation and oven rise (Gan et. al., 1995). The remaining constituents include all other ingredients that are added to dough, including yeasts, salt, malt, enzymes, flour improvements, sugar, fats, emulsifiers, milk or soy solids, mould inhibitors, or, in the case of sourdough LAB starters in the one of the mentioned forms.

Dough is composed of a continuous phase in which gas cells are dispersed. This continuous phase is called "dough phase" (Bloksma & Bushuk 1988). The following constituents can be distinguished:

1. Starch granules, occupying about 60% of the volume of the dough phase. Large elliptical granules occur side by side with small spherical granules. Size of this granule is significantly differing between normal wheat dough and sourdough (Brummer & Lorenz, 1991);
2. Swollen protein
3. Yeast cells, with the diameter of about 2 μm
4. LAB cells (in the case of sourdough)
5. Lipids
6. Irregularly shaped remnants of cell organelles and wheat grain tissues (Pomeranz, 1988). Immediately after mixing, the gas cells form spherical holes with diameters between 10 and 100 μm . Their number at that stage is estimated to be between 10^{11} and 10^{13} m^{-3} . Clarke et al. (2002) reported that LAB starters do not significantly influencing on gas transformation in dough, but certain differences between wheat dough and sourdough has been revealed by Hammes & Vogel (1995).

Wheat flour dough, same like sourdough, is viscoelastic, exhibits both flow and elastic recovery. When a piece of dough is placed on a flat surface, if the humidity is high enough so it will flow. The amount of flow depends on the balance of viscous and elastic properties (Faridi & Faubion, 1986). In fact, dough is not truly elastic such as rubber or similar artificial materials. If a piece of dough be stretched rapidly and the force be released immediately, it will only partially recover its original shape (Hoseney, 1994). In dough, the cross-links between molecules are secondary (noncovalent) bonds that are constantly breaking and reforming other units (Rao, 1984). This appears that starch is a not an inert ingredient in

flour-water systems. Instead starch appears to act as filler in the gluten polymer. Filled polymers are known to have a larger modulus than their unfilled counterparts.

In general, sourdough structure could be described similar to the model of the only yeast-fermented dough. However, according to all facts mentioned in previous text, it is so obvious that sourdough have different structure, due to added LAB starters (Koceva Komlenić et. al., 2010).

5.3 Fundamentals in dough rheology

Rheology is the study of how materials deform, flow, or fail when force is applied. If a material viscosity is constant regardless of stirring or flowing through pipe, these materials are called Newtonian. However, in many food systems, including flour-water systems, the viscosity changes (decreases) as the shear rate is increased (Bagley, 1992). These systems show more complicated non-Newtonian behaviour, and we cannot define the system by a single viscosity value, but must give viscosity at each shear rate. Additionally, viscosity can also be affected by the time involved in making the measurement (Hoseney, 1994). Many different kind of rheological moduli exist in applied rheology. In general, modulus refer to the stiffness of the material and is proportionally constant relating stress to strain. It tells how much force is required to produce a specific deformation of the material under test (Rao, 1986).

In cereal technology, farinograph, mixograph, extensograph and amylograph have been referred to as rheological measurements (Hoseney, 1994). These instruments measure how doughs deform and flow. The problem with the use of these instruments for rheological studies is that cannot define the stress at any moment of time during the test. This is not to say that above mentioned instruments are not useful. They have stood the test of time and can give much useful information. They are particularly used when used to characterize flour. It has been important to know whether the mixing properties of the flour used today are similar to or different from those of the flour used yesterday. The mixograph or farinograph can easily and rapidly detect this (Faridi & Faubion, 1986). Unlike, from fundamental measurements we can learn much more about rheological behaviour of dough. We can find effect of various interactions and how the properties of the dough change as a function of time or temperature. Additionally, the measurements can often be made on the complete dough system so the results are easy to interpret. The second reason is, that it has now become much easier to obtained good rheological data with the advent of minicomputers incorporated to the measurements and their related equipments (Hoseney, 1994).

Rheological measurements recently used for the characterization of dough rheology are described below.

5.4 Rheological tests on dough

The physical characteristics of doughs are important in relation to the uses of flours. In the case of sourdough, LAB fermentation is another factor. Pseudo-rheological characteristics are investigated mainly with the following methods:

1. The Brabender Farinograph measures and records the resistance of dough to mixing as it is formed from flour and water, developed and broken down. This resistance is called consistency. The maximum consistency of the dough is adjusted to a fixed value by

altering the quantity of water added. This quantity, the water absorption may be used to determine a complete mixing curve, the various features of which are guide to the strength of the flour (Figure 2)

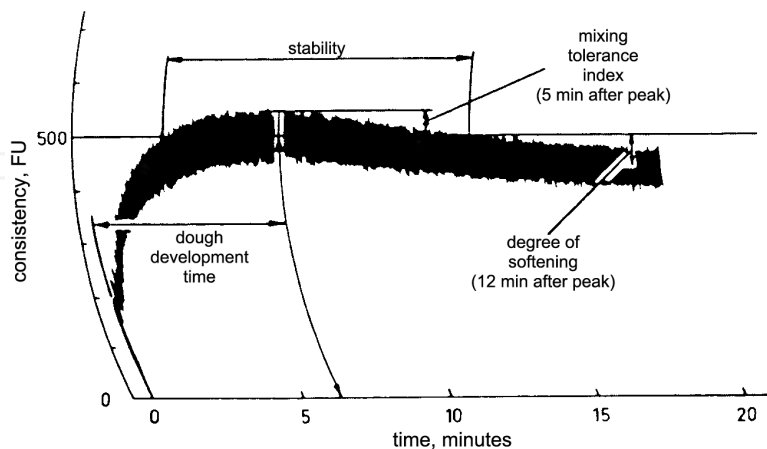


Fig. 2. Representative farinogram showing some commonly measured indexes. A consistency of 500 farinograph units (FU) corresponds to a power of 68 and 81 W per kilogram of dough in mixtures for 300 and 50 g of flour, respectively (Bloksma & Bushuk, 1988).

2. The Brabender Extensograph records the resistance of dough to stretching and the distance the dough stretches before breaking. A flour-salt-water dough is prepared under standard conditions in the Brabender Farinograph and moulded on the Extensograph into a standard shape. After a fixed period the dough is stretched and a curve drawn, recording the extensibility of the dough and its resistance to stretching (Fig. 3). The dough is removed and subjected to a further two stretches. The Extensograph has replaced the Extensometer in the Brabender instrument range.

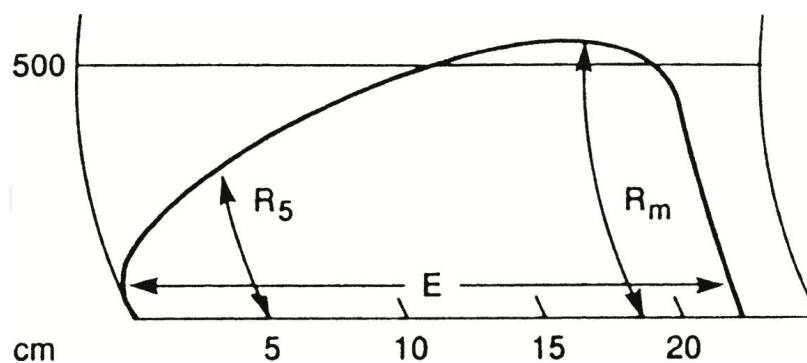


Fig. 3. Extensigram, showing extensibility (E), resistance at a constant extension of 5 cm (R_5) and maximum resistance (R_m). (Hoseney, 1994).

3. The Chopin Alveograph uses air pressure to inflate a bubble of dough until it bursts. The instrument continuously records the air pressure and the time that elapses before the dough breaks (Figure 4).
4. The Brabender Amylograph continuously measures the resistance to stirring of a 10% suspension of flour in water while the temperature of the suspension is raised at a constant rate of 1.5 °C/min from 20° - 95 °C. It is of use in testing flour for soups (etc.),

for which purpose the viscosity of the product after gelatinization is an important characteristic, as well as for adjusting the malt addition to flours for breadmaking

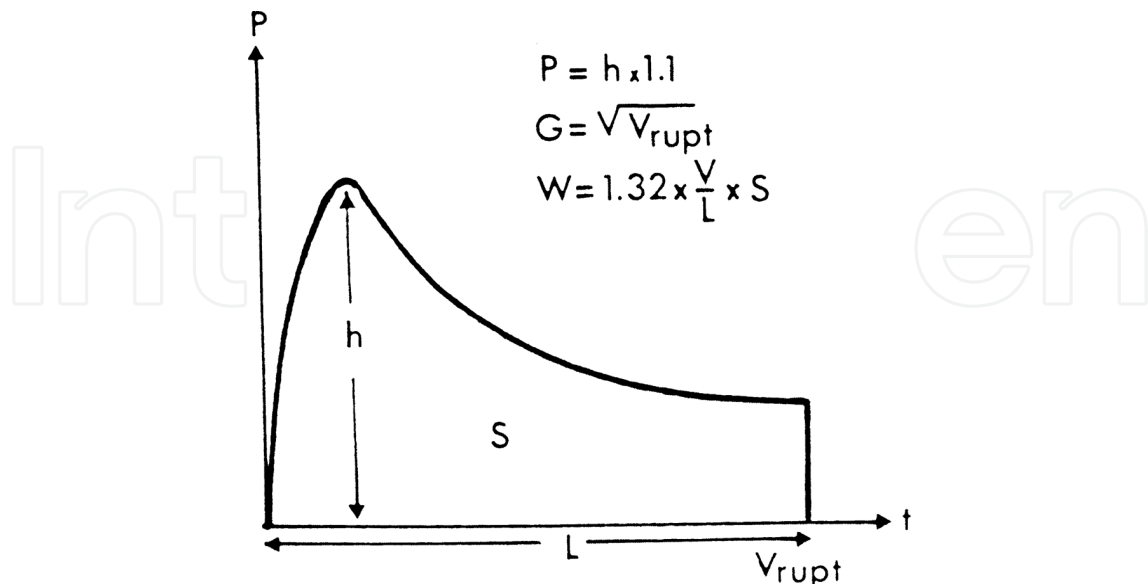


Fig. 4. A representative alveogram. P = overpressure (mm), L = abscissa at rupture (mm), G = swelling index (ml), V = volume of air (ml), W = deformation energy (10^{-4} J), h = maximum height (mm) and S = area under the curve (cm^2). (Hoseney, 1994).

5. The Rapid Visco Analyzer (RVA), may be regarded as a derivative of the Amylograph. Measurements of viscosity are made using small samples, containing 3-4 g of starch, in periods which may be as short as 2 min. Use of disposable containers and mixer paddles eliminates the needs for careful washing of the parts between tests. As with the Amylograph, the characteristics of starch pastes and the effects of enzyme of them can be recorded on charts. They can also be transferred in digital form direct to a data-handling computer.
6. True dynamic rheological instruments. In recent years frustration with instrument-dependent units obtained with some of the above methods, together with the poor reproducibility, from one instrument to another of the same type, has led cereal chemists to turn to true rheological measurements. Suitable instruments for use with doughs, slurries and gels, derived from flours, include Bohlin VOR (viscometric, oscillation and relaxation), the Carri Med CSL Rheometer and the Rheometrics RDA2. In addition to providing excellent reproducibility, these instruments, which are also used in many non-foods, and many non-cereal food materials, allow comparisons to be made across a wide range of substances. Although they are expensive, they will undoubtedly enable the development of tests that can be performed on simpler, dedicated instruments (Faridi & Faubion, 1990; Kent & Evers, 1994).

5.5 Documented rheological results on the sourdough: Recapitulation

In a number of previous studies differences between simple yeast-fermented doughs, chemically acidified (using lactic or acetic acid) doughs and biologically acidified (using a starter culture) dough were investigated by the use of empirical and dynamic rheology measurements (Zeng et. al., 1997; Wehrle et. al., 1997; Wehrle & Arendt, 1998; Lee et. al.,

2001; Clarke et. al. 2002; Clarke et. al., 2004; Angioloni et. al., 2006). Hosenev (1994) reported that acid developed during fermentation with LAB strongly influence the mixing behaviour of doughs, whereby doughs with lower pH values require a slightly shorter mixing time and have less stability than normal doughs. The water absorption of flour 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 (Caterall, 1998). Incorporation of sourdough changed the mixing behaviour, resulting in a significant decrease in water absorption relative to control (Maher Galal et. al., 1978; Wehrle et. al., 1997; Clarke et. al., 2002; Koceva Komlenić et. al., 2010). The addition of sourdough prepared with starter culture significantly reduced the stability of the dough relative to both the control (yeast fermented) and chemically acidified dough (Wehrle et. al., 1997; Clarke et. al., 2002; Clarke et. al., 2004). A large difference was found for the degree of softening. Addition of sourdough or acid significantly increased the degree of softening (Maher Galal et. al., 1978; Clarke et. al., 2002). Clark et. al. (2002) reported that biologically acidified doughs (sourdoughs) showed a significantly greater degree of softening than the chemically acidified (with lactic or acetic acid) doughs (Table 3. and Figure 5.).

Sample**	Water absorption (g/100g)	Dough development time (min)	Stability (min)	Degree of softening (BU)
Control I	62.15 ± 0.06 g	2.25 ± 0.29 e	2.03 ± 0.05 a	61.25 ± 2.50 e
LAB I	60.23 ± 0.05 h	3.25 ± 0.29 c	0.90 ± 0.23 e	82.50 ± 2.89 b
DS I	64.28 ± 0.05 e	2.80 ± 0.23 d	1.70 ± 0.12 b	66.25 ± 2.50 cd
LA I	62.35 ± 0.06 f	2.15 ± 0.17 e	1.40 ± 0.01 c	62.50 ± 2.89 de
Control II	67.75 ± 0.06 c	3.98 ± 0.05 b	1.28 ± 0.05 cd	65.00 ± 5.77 cde
LAB II	65.58 ± 0.05 d	4.63 ± 0.10 a	0.65 ± 0.06 f	122.50 ± 2.89 a
DS II	69.23 ± 0.05 a	4.20 ± 0.14 b	1.05 ± 0.29 e	81.25 ± 2.50 b
LA II	68.10 ± 0.12 b	4.03 ± 0.05 b	0.90 ± 0.23 e	67.50 ± 2.89 c

*Values are mean ± SD of four independent determinations; Mean values followed by common letter within the same column are not significantly different ($p < 0.05$)

**Control, control dough; I and II, flour types: T-550 and T-110, respectively; LAB, biologically acidified dough by addition of sourdough prepared with *Lactobacillus brevis* L-75; DS, biologically acidified dough by addition of dry sourdough; LA, chemically acidified dough by addition of lactic acid

Table 3. Farinogram results* for different wheat doughs (control, biologically and chemically acidified doughs). (Koceva Komlenić et al. 2010).

The effect of sourdough addition on extensibility of dough were also investigated (Tsen, 1966; Corsetti et. al., 2000; Clarke et. al., 2002; Katina et. al. 2006a; Katina et. al. 2006b, Arendt et. al., 2007; Koceva Komlenić et. al., 2010). The Extensograph gives information about about a dough extensibility and resistance to extension (Walker & Haazelton, 1996). Generally, the addition of sourdough or acid significantly reduced extensibility of the dough. Clarke et. al. (2002) demonstrated the higher reduction of extensibility when the mixed-strain starter culture was used compared to single strain used (Figure 6). It is in agreement with the results obtained by Tsen (1966).

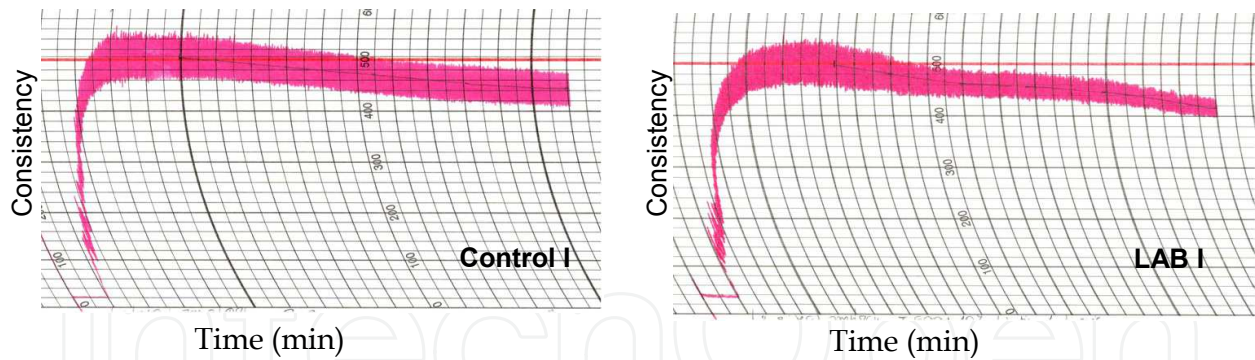


Fig. 5. Farinograms of doughs: Control I (prepared with flour type I) and LAB I (prepared with flour type I and *L. brevis* preferment). (Koceva Komlenić et al. 2010).

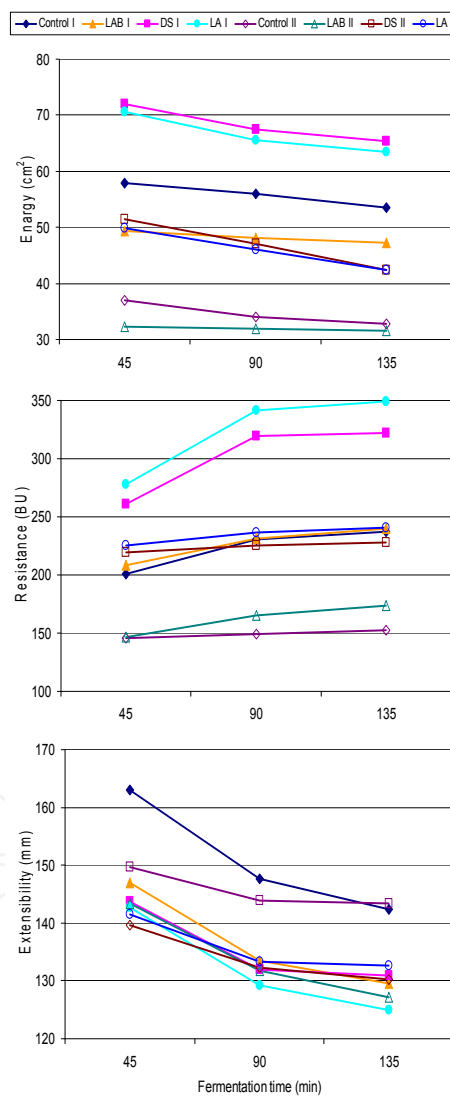


Fig. 6. Extensograph analysis of doughs with sourdough and lactic acid: I and II (different flour type), LAB (biologically acidified dough by addition of sourdough prepared using a starter culture of *L. brevis* L-75), DS (biologically acidified dough by addition of dry sourdough), LA (chemically acidified dough by addition of lactic acid). (Koceva Komlenić et al. 2010)

Dynamic oscillatory measurements examined the effects of sourdough or acid addition on the viscoelastic properties of the dough. The rheological characteristics of fermented dough are determined by many factors. At the beginning of the mixing process, physical actions, such as hydration, take place. The gluten network is formed by proteins, and starch granules absorb water. Enzyme activity of amylases, proteases and hemicellulases causes breakdown of several flour components. Changes of pH level also alter the rheological behaviour of the dough (Wehrle et al. 1997). Even small chemical and physical changes in gluten network can lead to significant changes in rheological characteristics. Formation of gas during fermentation of sourdough leads to an increased volume and decreased density.

According to the most of the authors, main variables of viscoelastic properties of the dough are phase angle (δ) and complex modulus G^* (Figure 7 and 8.). The results of fundamental oscillatory measurements gave the key attributes of rheological behaviour of the dough. The addition of sourdough prepared by LAB starters did increase the phase angle values relative to the control or the chemically acidified dough significantly (Clarke et al., 2002). It means that addition of sourdough reduced the elasticity of the dough in contrast to the chemical acidification, which did not have the same effect (Hoseney, 1994). Some authors observed that there were no significant differences between G^* values for the control and chemically acidified doughs over all frequencies, while the values for the control were significantly higher than those obtained from sourdough over all frequencies. The same was true for the chemically acidified doughs which yielded greater G^* values than the sourdoughs. An increase in phase angle and decrease in G^* due to addition of sourdough indicated that the dough was less elastic and became simultaneously less firm at low rate of strain applied (0.1%). These results also clearly suggest that chemical acidification was not directly comparable with biological acidification. Thiele et al. (2002) reported that, with regard to the levels of amino acids in wheat dough, may influence on sourdough rheology. Clarke et al. (2004) also reported a dramatic loss of firmness and elasticity in preferments during 24-hours fermentation of sourdough. These results suggest that cereal proteases with acidic optima play a central role in the rheological changes taking place during sourdough fermentation.

Wehrle & Arendt (1998) analyzed rheological characteristics of controlled and spontaneous fermented sourdough. During the first 5 hours of fermentation, phase angles did not change significantly. After 5 hours phase angles of sourdough increased sharply. Maximum phase angles were achieved after fermentation time of 20 hours. A high phase angle indicates viscous behaviour of material. Fermented sourdough after 20 hours of fermentation is an entirely viscous manner, with phase angles close to 90° , which indicates an ideal fluid. Phase angle was closely related to CO_2 production by heterofermentative microorganisms in the dough. Results of Wehrle & Arendt indicate the beginning of gas production in the sourdough after 5th hour of fermentation (Table 4). Clarke et al. (2004) also observed a dramatic loss of firmness and elasticity during 24-hr period irrespective of the presence of acid or lactic acid bacteria. The presence of acid, however, enhanced the effects. Wehrle et al. (1997) reported that doughs with acid initially were firmer and more viscous than doughs without acids, but showed less stability during mixing. Addition of acids intensified decrease in G^* and increase in δ with longer mixing times when measured immediately after mixing.

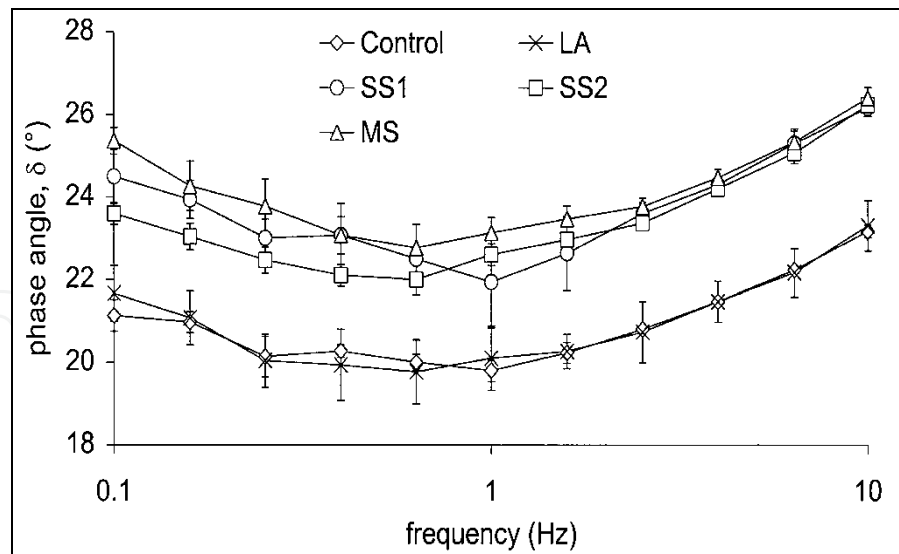


Fig. 7. Phase angle (δ) as a function of frequency for wheat dough formulations: control (nonacidified), LA (chemically acidified with lactic acid), SS1 (added sourdough prepared using a single strain starter culture of *Lactobacillus brevis* L-62), SS2 (added sourdough prepared using a single strain starter culture of *L. plantarum* L2-1), MS (added sourdough prepared using a mixed strain starter culture, Böcker Reinzucht-Sauerteig Weizen). Mean value \pm standard deviation of three replicates (Clarke et al. 2002).

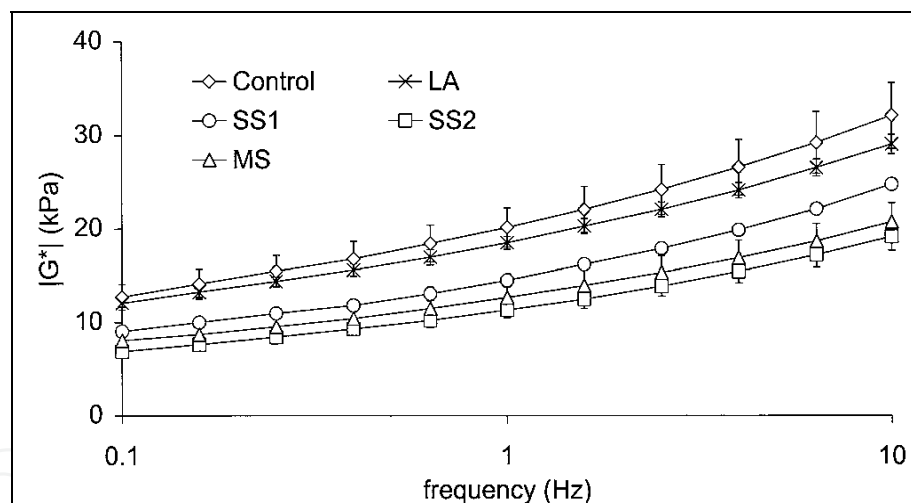


Fig. 8. Absolute value of the complex dynamic modulus ($|G^*|$) as a function of frequency for wheat dough formulations: control (nonacidified), LA (chemically acidified with lactic acid), SS1 (added sourdough prepared using a single strain starter culture of *Lactobacillus brevis* L-62), SS2 (added sourdough prepared using a single strain starter culture of *L. plantarum* L2-1), MS (added sourdough prepared using a mixed strain starter culture, Böcker Reinzucht-Sauerteig Weizen). Mean value \pm standard deviation of three replicates. (Clarke et al. 2002).

Rheological properties, as well as acidification and flavour development, are important parameters in controlling fermentation process. Types of microorganisms, metabolic activity, and time-dependent development of pH levels have an effect on the final rheological properties.

	Control	LA ^b	SS1 ^c	SS2 ^d	MS ^e
Dough development curve parameters					
Hm ^f (mm)	87.0 ± 1.0b	79.0 ± 2.6ab	75.2 ± 1.2a	76.0 ± 3.3a	87.8 ± 6.3b
h ^g (mm)	86.3 ± 0.4b	78.3 ± 3.8ab	71.8 ± 2.3a	72.7 ± 3.6a	87.3 ± 6.9b
(Hm - h)/Hm ^h (%)	0.7 ± 0.8ab	0.9 ± 1.6abc	4.5 ± 2.1c	4.3 ± 0.8bc	0.6 ± 0.7a
T1 ⁱ (min)	177 ± 3b	179 ± 2b	140 ± 22a	132 ± 6a	179 ± 1b
Gaseous release curve parameters					
T1 ^j (min)	114.0 ± 9.1b	117.0 ± 4.0b	76.5 ± 10.4a	96.0 ± 21.3ab	67.0 ± 5.7a
Vt ^k (mL)	1,942 ± 50ab	2,019 ± 56b	1,986 ± 52ab	2,021 ± 16b	1,870 ± 75a

^a Mean value ± standard deviation of two replicates. Mean values followed by a common letter within the same row are not significantly different ($P < 0.05$).

^b Chemically acidified by the addition of lactic acid.

^c Added sourdough prepared using a single strain starter culture of *Lactobacillus brevis* L-62.

^d Added sourdough prepared using a single strain starter culture of *L. plantarum* L2-1.

^e Added sourdough prepared using a mixed strain starter culture, Böcker Reinzucht-Sauerteig Weizen.

^f Maximum height of dough development curve.

^g Dough development height at the end of 3-hr test period.

^h % Reduction in dough development height at the end of test period relative to T1.

ⁱ Time of maximum height of dough development curve.

^j Time of maximum height of gaseous release curve.

^k Total volume of carbon dioxide released by dough.

Table 4. Development and Gaseous Release Characteristics of Doughs from Rheofermentometer Testinga (Clarke et al. 2002)^a

6. Perspectives

The composition and processing of cereals grains, substrate formulation, growth capability and productivity of starter culture, stability of probiotic strains during storage, organoleptic properties and nutritional value of the final product are key parameters to be considered (Charalampopoulos, 2002). Probiotics bacteria from the sourdough starters must meet the criteria not only for good survival during fermentation process but also for fermentation and symbiosis with other starter cultures used (Kedia et. al., 2007). Nowadays, the use of probiotic bacteria as starter cultures in baked goods is at its early stage and merits further investigation (Font de Valdes et. al., 2010). Since sourdough systems are so complex, and the determination of rheological properties of dough should be comprehensive to reveal influence of sourdough composition and all components of flour (gluten, starch, lipids, water soluble proteins and pentosans). Regardless of whether the empirical or fundamental rheological testing is used and despite the decades of bread dough rheology testing there is still no universal equipment or method that can provide the data that are 100% accurate in evaluating performance during dough processing. Over time, numerous models of dough rheological behavior have been developed to simplify describing the rheological changes in bread dough system and they are still emerging.

Recently, Tanner *et al.* (2008) introduced the use of a simple Lodge-type model (Lodge, 1964), including a power law memory function, and a damage function (assumed to be a function of strain) to represent the breakdown of the molecular structure within the dough, for a description of the rheological behavior of wheat dough. The model produced a

satisfactory reconstruction of stress data in shear start up and elongation flow at low deformation rates. The model has relatively few parameters, all of which can easily be found from simple experiments. The sequence of step strains, with both reversing and steadily increasing steps, is reasonably well described by the model. The model can therefore describe small-strain motions, steady shearing, steady uniaxial and biaxial elongations, recoil, stress relaxation and step strain motions. Since then, several modifications of this model have been developed. Hicks *et al.* (2011) find that the damage term is explicitly a function of strain, a concept that may carry over to elongation flow and that rapid decrease of the damage function post material fracture is a representation of the transition of dough from solid- to liquid-like rheological behavior. Tanner *et al.* (2011) described an improved damage function model for bread dough rheology that enables describing uniaxial and biaxial stretching with the same damage function derived from shear data.

Further investigations of dough rheological behavior is required to obtain quality results and to develop models that accurately simulate processing situations and parameters and provides a platform for comprehensive process control.

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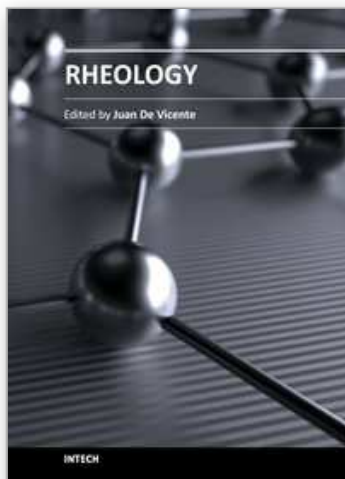
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