

# Effect of Single Strain and Traditional Mixed Strain Starter Cultures on Rheological Properties of Wheat Dough and on Bread Quality

C. I. Clarke,<sup>1,2</sup> T. J. Schober,<sup>1,2</sup> and E. K. Arendt<sup>1,3</sup>

## ABSTRACT

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Investigations were made to test the effect of two different sourdough starter culture types on wheat dough and bread quality. Two single-strain starter cultures consisting of well-defined strains of lactic acid bacteria (*Lactobacillus plantarum*, *L. brevis*) and a traditional mixed-strain sourdough culture (containing *L. crispatus*, *L. pontis*, and *Saccharomyces cerevisiae*) were evaluated for their effects on the rheological characteristics of wheat dough using both fundamental rheological and standard baking tests. Two other doughs were also evaluated, one which was chemically acidified to a comparable pH value by the addition of lactic acid, and a control which was not acidified. Dynamic oscillation tests were per-

formed using a controlled stress rheometer. The phase angle and the absolute value of the complex dynamic modulus were measured for all doughs at frequencies of 0.1–10 Hz. The addition of sourdough prepared using single-strain or mixed-strain cultures significantly increased the phase angle and reduced the complex modulus of the doughs at all frequencies ( $P < 0.05$ ). Significant differences were found between the dough which was chemically acidified and those doughs which were biologically acidified. The addition of sourdough effected an increase in loaf specific volume relative to both the chemically acidified and the non-acidified doughs.

Sourdough fermentation is one of the oldest biotechnological processes used in food production and indeed it was the only bread leavening method used before the discovery of yeast in beer production (Röcken and Voysey 1995). The acidification process effected by the use of sourdoughs remains a necessary prerequisite for the preparation of rye breads or rye-wheat combination breads (Hammes and Gänzle 1998). The application of sourdough to wheat breads, meanwhile, has regained importance as a means to improve the quality and flavor of wheat breads (Stear 1990; Brümmer and Lorenz 1991; Corsetti et al 2000; Thiele et al 2002). It has also been reported that the use of sourdough can have positive nutritional implications by increasing mineral bioavailability (Salovaara and Goransson 1983; Larsson and Sandberg 1991) and by lowering the glycaemic response to baked goods (Liljeberg and Björck 1994; Liljeberg et al 1995). More recent studies have confirmed the positive effect of sourdough antifungal compounds on the mold-free shelf life of baked goods (Lavermicocca et al 2000; Magnusson and Schnürer 2001).

The incorporation of sourdough during wheat bread production can have a considerable effect on the characteristics of the dough. The effects are complex due to variation between sourdoughs with regard to the type of starter culture, dough yield, and fermentation regime used (Wehrle et al 1997). Many of the effects of sourdough have been attributed to a drop in pH value caused by the production of organic acids. It is acknowledged, however, that the fermentation process employed does considerably more to influence the properties of the dough than simply produce acid (Wood et al 1975; Hammes and Gänzle 1998; Thiele et al 2002). This may be due to the complex series of interactions that take place within the dough system. Dough is very sensitive to changes in ionic strength and pH, and such changes may have a direct impact on the constituents responsible for the development of dough structure such as gluten, starch, and hemicelluloses. Changes in the environmental pH may also act indirectly on the flour enzymes present within the dough system, thus influencing the nature and degree of proteolytic and amylolytic activity that takes place (Thiele et al 2002). Furthermore, the sourdough organisms may also contribute to changes in the dough structure depending on their characteristic properties such as

specific enzymatic activities (Gobbetti et al 1996) or the production of substances such as exopolysaccharides (Korakli et al 2001).

Sourdoughs have been grouped into three types based on the technology used for their production (Böcker et al 1995; Hammes and Gänzle 1998). Type I doughs typically employ a multiple-stage fermentation process and the microflora are sustained by repeated inoculation. Strains of *Lactobacillus sanfranciscensis* are the predominant microflora in rye and wheat sourdoughs of this type, while *L. pontis* is present to a lesser extent. Portions of this sourdough are used as inocula and these may be defined as “multiple strain starter cultures” (Hammes 1991). Type II doughs, meanwhile, serve to provide acidification and flavor only, while leavening is performed by added baker’s yeast. They have a higher water content and are fermented for a longer time using elevated temperatures. Vogel et al (1999) have documented that such doughs are mostly used to produce bakery preproducts in industrialized processes. Dried preparations of sourdough are as type III. The lactic acid bacteria in these doughs are resistant to drying and include *L. plantarum* and *L. brevis*.

A number of studies have examined the influence of acids and varying pH values on the properties of dough or its constituents. Using empirical rheological measurements, Maher Galal et al (1978) reported that the addition to dough of a combination of organic acids alone increased water absorption, while dough development time, stability, and tolerance to mixing were decreased. A strong inter-

TABLE I  
Bread Dough Recipes<sup>a</sup>

	Control	LA <sup>b</sup>	SS1 <sup>c</sup>	SS2 <sup>d</sup>	MS <sup>e</sup>
Flour	1,000	1,000	800	800	800
Water <sup>f</sup>	610	609	390	390	395
Salt	20	20	20	20	20
Yeast <sup>g</sup>	15	15	15	15	15
Lactic acid	...	5.5	...	...	...
Sourdough <sup>h</sup>	...	...	400	400	400
Total H <sub>2</sub> O in formulation	610	609	590	590	595

<sup>a</sup> Quantities based on 1 kg of flour.

<sup>b</sup> Chemically acidified by the addition of lactic acid.

<sup>c</sup> Added sourdough prepared using a single strain starter culture of *Lactobacillus brevis* L-62.

<sup>d</sup> Added sourdough prepared using a single strain starter culture of *L. plantarum* L2-1.

<sup>e</sup> Added sourdough prepared using a mixed strain starter culture, Böcker Reinzucht-Sauerteig Weizen.

<sup>f</sup> Water addition for each formula was based on % farinograph water absorption.

<sup>g</sup> Instant active dried yeast.

<sup>h</sup> Sourdough had a dough yield of 200 (1 part water and 1 part flour).

<sup>1</sup> Department of Food Science, Food Technology and Nutrition, National University of Ireland, Cork, Ireland.

<sup>2</sup> National Food Biotechnology Centre, National University of Ireland, Cork, Ireland.

<sup>3</sup> Corresponding author. E-mail: e.arendt@ucc.ie. Phone: +353-21-4902064. Fax: +353-21-4270213.

action was found between salt and acid: the addition of acids together with 1.5% salt (fwb) greatly increased dough development time, whereas dough stability was increased to a lesser extent. Also using empirical rheological measurements in the form of extensograms, Tsen (1966) reported that a reduction in dough pH from 5.8 to 5.2 or 4.8 by the addition of HCl decreased extensibility. Wehrle et al (1997) used both farinograms and fundamental rheological measurement techniques to evaluate the effects of a combination of lactic acid and acetic acid, the main metabolites of the sourdough process, on dough properties. These authors found that doughs containing acid in the absence of salt were characterized by an increased phase angle and reduced complex modulus indicative of overmixing. Little information is available, however, on the effects of the incorporation of biologically acidified sourdough on the fundamental rheological properties of wheat dough. The present study builds on the approach used by Wehrle and Arendt (1998) by comparing biological and chemical acidification processes. The results obtained from fundamental rheological and baking tests are also compared.

## MATERIALS AND METHODS

### Materials

Commercial breadmaking wheat flour with a protein content of 12.4% (dry basis), 0.5% ash content (dry basis), 25 ppm of ascorbic acid, and a Hagberg falling number value of 250 (Odlum Group, Dublin, Ireland) was used in the test series. Potable water was used for baking tests and distilled water was used for rheological tests. Instant dried yeast (Mauripan) was supplied by Burns Philip Food Ltd. (Lasalle, QC H8R IZ8). Vacuum-dried salt (Salt Union, Weston Point, Runcorn, Cheshire, UK) was also incorporated into the dough. The lactic acid used was an analytically pure chemical from British Drug House (Poole, UK). A commercial mixed strain starter culture Böcker Reinzucht-Sauerteig Weizen (BRSW) specifically designed for use in wheat sourdough was used (Böcker, Minden, Germany). The starter is based on a coarse-grain material that contains a high number of living bacteria cells, including four *Lactobacillus* strains (one *L. crispatus* and three *L. pontis*) ( $\approx 10^9$  CFU  $g^{-1}$ ) and a strain of *Saccharomyces cerevisiae* ( $\approx 8 \times 10^6$  CFU  $g^{-1}$ ). Two freeze-dried single-strain starter cultures were also used to prepare sourdough, a heterofermentative starter culture, *L. brevis* L-62 ( $\approx 2 \times 10^{11}$  CFU  $g^{-1}$ ) (Chr. Hansen, 2970 Hørsholm, Denmark) and a facultatively heterofermentative but typically homofermentative starter culture, *L. plantarum* L2-1 ( $\approx 1 \times 10^{11}$  CFU  $g^{-1}$ ) (Danisco Cultor GmbH, 25899 Niebüll, Germany).

### Sourdough Preparation

Sourdough was prepared from 10% (fwb) mixed-strain starter or 0.01% (fwb) single-strain starter, due to the different cell counts of the starter material. The starter was added to equal parts of water and wheat flour, thereby giving a dough yield (DY) of 200. The coarse-grain mixed strain starter was homogeneously dispersed in

the flour and the single-strain freeze-dried starter was dispersed in the water. Dough was mixed thoroughly for 1 min, poured into a large beaker, covered, and placed in an incubator (Mettmert GmbH & Co., Schwabach, Germany) at 30°C for 20 hr. The total titratable acidity and pH values of the sourdough were determined in a suspension of sourdough (10 g), acetone (5 mL), and distilled water (95 mL) according to a standard method (Arbeitsgemeinschaft Getreideforschung e.V. 1994).

Table I shows the dough recipes. The control dough was prepared with flour, water, yeast, and salt. The chemically acidified dough (LA) contained, in addition, lactic acid at a rate of 0.55% (fwb) to yield a dough with a pH value comparable with that of the biologically acidified doughs. The sourdoughs were produced by replacing 20% of the flour with an equivalent quantity of flour in the form of sourdough from either of the single strain (SS) starter cultures, *L. brevis* L-62 (SS1) and *L. plantarum* L2-1 (SS2), or from the mixed strain starter culture, BRSW (MS).

### Physical Measurements

Farinograph (Brabender OHG, Duisburg, Germany) measurements were made using a 300-g mixing bowl and a mixing speed of  $63 \pm 2 \text{ min}^{-1}$  according to a modified version of a standard method (ICC 1996). The modification included the addition of sourdough premixed with flour at the beginning of the mixing period before the addition of water. Each result is the average of three measurements.

Doughs containing flour, water, salt, and added sourdough were tested with the Extensograph (Brabender, Duisburg, Germany) according to a standard method (ICC 1996). Each result is the average of six measurements (three individual doughs each measured in duplicate).

Doughs for rheological measurement were prepared in a Glutomatic 2200 (Perten Instruments AB, Huddinge, Sweden), which allowed constant and reproducible mixing of small quantities of dough. Mixing speed was 120 rpm. As the Glutomatic is normally used to determine the gluten content according to a standard method (ICC 1996), normal addition of washing solution was stopped, and a modified mixing chamber without a bottom perforation was used to mix the dough for 70 sec. Doughs were prepared with 10 g of flour, water, and added sourdough or lactic acid. After mixing, the doughs were gently removed from the mixing bowl, scaled to a 5-g portion and allowed to rest in a covered petri dish for 15 min. The rested dough sample was placed between the rheometer plates, excess dough protruding from the edge of the plate was carefully trimmed, and the whole system was covered. Water-saturated cotton strips were placed on the inner side of the cover to create an atmosphere with high relative humidity to prevent drying out of the dough rim. Therefore, no drying out of the dough rim was discernable during the tests. Before commencing the oscillatory measurement, the dough was rested for 5 min, allowing normal stresses induced during sample loading to relax.

TABLE II  
Farinograph Mixing Characteristics of Doughs<sup>a</sup>

	Control	LA <sup>b</sup>	SS1 <sup>c</sup>	SS2 <sup>d</sup>	MS <sup>e</sup>
Absorption (%)	61.0 ± 0.0c	60.9 ± 0.1c	59.0 ± 0.0a	59.0 ± 0.0a	59.5 ± 0.0b
DDT <sup>f</sup> (min)	2.5 ± 0.1a	2.6 ± 0.4ab	3.1 ± 0.1ab	3.0 ± 0.3ab	3.1 ± 0.2b
Stability (min)	5.6 ± 0.8b	4.3 ± 0.4b	2.4 ± 0.4a	2.6 ± 0.8a	2.4 ± 0.5a
E10 <sup>g</sup> (BU)	40.0 ± 0.0a	55.0 ± 5.0b	73.3 ± 5.8c	70.0 ± 0.0c	80.0 ± 5.0c
E20 <sup>h</sup> (BU)	88.3 ± 2.9a	116.7 ± 2.9b	143.3 ± 7.6d	131.7 ± 2.9cd	123.3 ± 5.8bc

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

<sup>b</sup> Chemically acidified by the addition of lactic acid.

<sup>c</sup> Added sourdough prepared using a single strain starter culture of *Lactobacillus brevis* L-62.

<sup>d</sup> Added sourdough prepared using a single strain starter culture of *L. plantarum* L2-1.

<sup>e</sup> Added sourdough prepared using a mixed strain starter culture, Böcker Reinzucht-Sauerteig Weizen.

<sup>f</sup> Dough development time.

<sup>g</sup> Degree of softening after 10 min.

<sup>h</sup> Degree of softening after 20 min.

Rheological measurements were performed on a controlled stress rheometer (CS-50, Bohlin Instruments Ltd., Cirencester, UK). The geometry consisted of parallel plates with a 40-mm diameter and a 2-mm gap. A strain sweep test was used to identify the linear viscoelastic region. A frequency of 10 Hz was used and the strain was varied from  $10^{-4}$  to  $10^{-1}$ . On the basis of this data, a target strain of  $10^{-3}$  (0.1%), which was within this linear region, was chosen for measurements. This value is in agreement with the finding of other researchers who employed values of 0.2–0.8%, as detailed by Campos et al (1997). A frequency sweep was used to characterize the doughs. Oscillation frequencies were 0.1–10 Hz (on a logarithmic scale) and 11 measuring points were recorded. The temperature was kept constant at 25°C. The Bohlin software package was used to calculate the absolute value of the complex dynamic modulus ( $|G^*|$ ) and the phase angle ( $\delta$ ). Every result is the average of at least three measurements.

The gaseous release and development characteristics of the dough were measured using a rheofermentometer (Chopin S.A., Villeneuve-La-Garenne, France). The dough was prepared in the same manner as

for baking. A 300 g quantity of dough and a 1,500 g cylindrical weight were used. The test was conducted over 3 hr at 30°C. A number of characteristics were determined from the gaseous release and dough development curves produced, the details of which have been previously described (Gobbetti et al 1995).

### Baking Tests

Doughs based on a flour quantity of 3,500 g (Table I) were mixed in a 30-quart capacity planetary mixer (Hunt 30, John Hunt Ltd., Bolton, Lancashire BL3 5BZ, UK) with a dough hook for 1 min at a disk speed of 44 rpm (shaft 88 rpm) and 7 min at a disk speed of 135 rpm (shaft 270 rpm). Water temperature was varied to yield a final dough temperature of 27–30°C. The dough was rested in bulk for 20 min in the proofer (Koma BV Roremond, Netherlands) at 30°C and 85% rh, scaled into 400-g portions, molded in a small-scale molder (Machinefabriek Holtkamp B.V., Almelo, Holland), placed in tins 180 mm × 120 mm × 60 mm (Sasa UK Ltd., Enfield, Middx EN3 7UL, UK) and proofed at 30°C and 85% rh for 60 min. Baking was at 230°C top heat and 230°C bottom heat

TABLE III  
Acidification Properties of Sourdoughs, Bread Doughs, and Breads<sup>a</sup>

	Sourdough		Bread Dough		Bread	
	pH	TTA <sup>b</sup>	pH	TTA	pH	TTA
Control	...	...	6.23 ± 0.08b	2.05 ± 0.07a	5.93 ± 0.01b	2.75 ± 0.21a
LA <sup>c</sup>	...	...	5.31 ± 0.04a	3.80 ± 0.07b	5.22 ± 0.13a	4.55 ± 0.35b
SS1 <sup>d</sup>	4.0 ± 0.00a	8.5 ± 0.18a	5.37 ± 0.06a	3.53 ± 0.18ab	5.23 ± 0.11a	4.58 ± 0.11b
SS2 <sup>e</sup>	4.1 ± 0.00a	8.2 ± 0.35a	5.43 ± 0.06a	3.60 ± 0.14ab	5.27 ± 0.02a	4.65 ± 0.21b
MS <sup>f</sup>	4.3 ± 0.28a	9.7 ± 2.97a	5.50 ± 0.23a	4.00 ± 0.85b	5.43 ± 0.19a	4.45 ± 0.21b

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

<sup>b</sup> Total titratable acidity (TTA) measured as mL of NaOH (0.1N)/10 g of sourdough, bread dough, or bread.

<sup>c</sup> Chemically acidified by the addition of lactic acid.

<sup>d</sup> Added sourdough prepared using a single strain starter culture of *Lactobacillus brevis* L-62.

<sup>e</sup> Added sourdough prepared using a single strain starter culture of *L. plantarum* L2-1.

<sup>f</sup> Added sourdough prepared using a mixed strain starter culture, Böcker Reinzucht-Sauerteig Weizen.

TABLE IV  
Development and Gaseous Release Characteristics of Doughs from Rheofermentometer Testing<sup>a</sup>

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

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

<sup>b</sup> Chemically acidified by the addition of lactic acid.

<sup>c</sup> Added sourdough prepared using a single strain starter culture of *Lactobacillus brevis* L-62.

<sup>d</sup> Added sourdough prepared using a single strain starter culture of *L. plantarum* L2-1.

<sup>e</sup> Added sourdough prepared using a mixed strain starter culture, Böcker Reinzucht-Sauerteig Weizen.

<sup>f</sup> Maximum height of dough development curve.

<sup>g</sup> Dough development height at the end of 3-hr test period.

<sup>h</sup> % Reduction in dough development height at the end of test period relative to T1.

<sup>i</sup> Time of maximum height of dough development curve.

<sup>j</sup> Time of maximum height of gaseous release curve.

<sup>k</sup> Total volume of carbon dioxide released by dough.

TABLE V  
Volume and Crumb Grain Characteristics of Breads<sup>a</sup>

	Control	LA <sup>b</sup>	SS1 <sup>c</sup>	SS2 <sup>d</sup>	MS <sup>e</sup>
Loaf specific volume (mL/g)	4.06 ± 0.01	4.29 ± 0.09	4.63 ± 0.39	4.56 ± 0.30	4.75 ± 0.30
Total number of cells	1,046 ± 15	1,116 ± 5	1,167 ± 50	1,159 ± 14	1,141 ± 29
Number of cells < 4 mm <sup>2</sup>	997 ± 16	1,074 ± 5	1,132 ± 62	1,122 ± 18	1,097 ± 27

<sup>a</sup> Mean value ± standard deviation of two replicates.

<sup>b</sup> Chemically acidified by the addition of lactic acid.

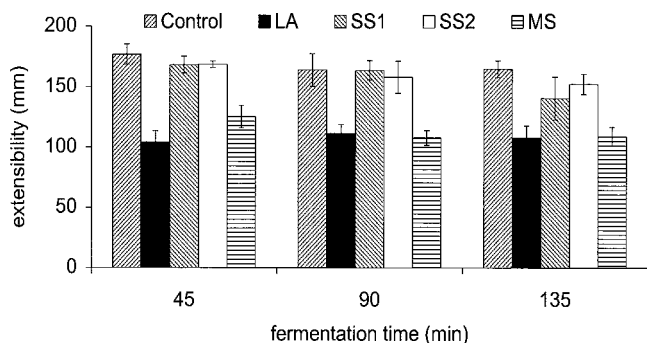
<sup>c</sup> Added sourdough prepared using a single strain starter culture of *Lactobacillus brevis* L-62.

<sup>d</sup> Added sourdough prepared using a single strain starter culture of *L. plantarum* L2-1.

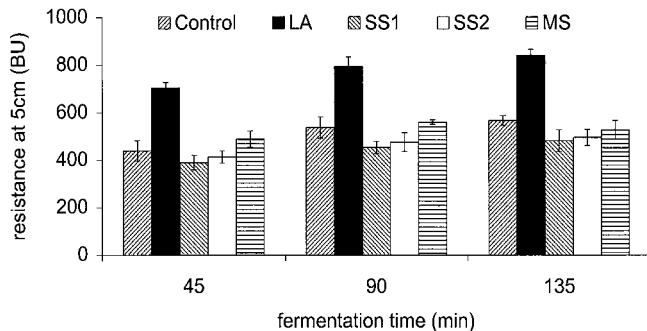
<sup>e</sup> Added sourdough prepared using a mixed strain starter culture, Böcker Reinzucht-Sauerteig Weizen.

for 30 min in a deck oven (MIWE, Arnstein, Germany). The oven was presteamed (0.3 L of water) before loading and, on loading, was steamed by injecting 0.7 L of water. The loaves were depanned and allowed to cool for 120 min on cooling racks at room temperature. Individual loaves were heat-sealed in moisture impermeable bags and stored at 21°C.

Three loaves were used for each analysis. Analyses were performed over a four-day storage period at 24-hr intervals, 2 hr (after cooling and before packing), 26 hr, 50 hr, and 74 hr. Loaf volume was measured using the seed displacement method and loaf weight was also recorded. Three determinations were made for each batch and averaged. Loaf specific volume (mL/g) was calculated from this data. Bread was sliced transversely using a slice regulator and bread knife to obtain uniform slices of 25 mm thickness. Instrumental textural evaluation of the crumb was made using a universal testing machine (TA-XT2i Texture Analyser, Stable Micro Systems, Godalming, Surrey, UK) equipped with a 25-kg load cell and a 35-mm aluminium cylindrical probe. A test speed of 2.0 mm/sec with a trigger force of 20 g was used to compress the central area of the bread slice to 40% of its original height. Each sample was compressed twice in a reciprocating motion to give a two-bite texture profile curve. A range of values for textural attributes was extracted from the resulting curve (Bourne 1978). Two bread slices taken from the center of each loaf were evaluated in this manner. The total titratable acidity and pH values of the breads were determined following the same standard method



**Fig. 1.** Extensigraph extensibility 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 six replicates.



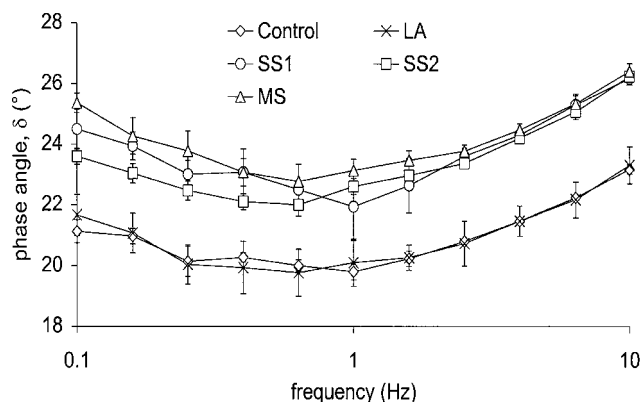
**Fig. 2.** Extensigraph resistance of dough at 5 cm extension for wheat dough formulations: control (nonacidified), LA (chemically acidified with lactic acid), SS1 (added sourdough prepared using a single strain starter culture of *Lactobacillus brevis* L-62), SS2 (added sourdough prepared using a single 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 six replicates.

described for sourdough (Arbeitsgemeinschaft Getreideforschung e.V. 1994). The crumb grain of the loaves was assessed using a previously described digital image analysis system (Crowley et al 2000), whereby a number of crumb grain features were determined to generate a crumb grain profile for each bread type. The crumb grain features determined included the total number of cells and the number of small cells (< 4 mm<sup>2</sup>) and large cells (> 4 mm<sup>2</sup>).

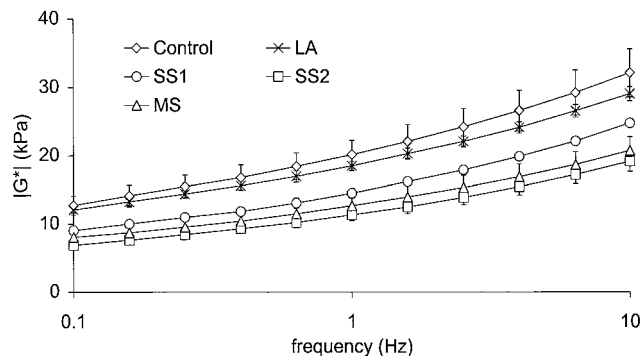
### Statistical Design and Analysis

Statistical analyses were performed using SPSS 10.0.5 for Windows computerized statistical analysis package (SPSS Inc., Chicago, IL). Farinograph, extensigraph, oscillation measurement, and rheofermentometer data were examined using one-way analysis of variance (ANOVA). Where an *F*-test showed significant differences (*P* < 0.05), Tukey's honestly significant difference (HSD) test was used for multiple comparison.

A blocked split-plot design was used for the baking. In this design, each trial was divided into five main plots, with the five formulations assigned randomly to these main plots. Subsequently, each main plot was divided into four smaller plots (split plots) for storage times (2, 26, 50, and 74 hr) which were then assigned to these plots (Mead and Curnow 1983). Each block was replicated once.



**Fig. 3.** Phase angle ( $\delta$ ) 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.



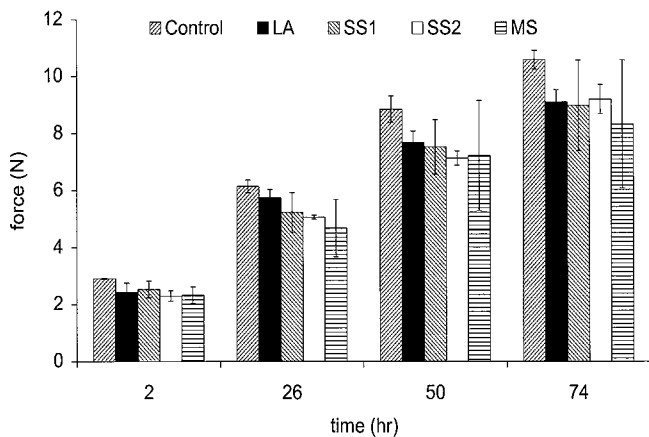
**Fig. 4.** Absolute value of the complex dynamic modulus ( $|G^*|$ ) as a function of frequency for wheat dough formulations: control (nonacidified), LA (chemically acidified with lactic acid), SS1 (added sourdough prepared using a single strain starter culture of *Lactobacillus brevis* L-62), SS2 (added 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.

## RESULTS AND DISCUSSION

### Farinograph

The farinograph is commonly used to provide empirical information regarding the mixing properties of dough (Spies 1990). 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 (Catterall 1998).

The rate of water addition was varied to give a standardized maximum dough consistency of 500 BU in accordance with the standard method (ICC 1996). The characteristic parameters of the farinograms obtained are detailed in Table II. Incorporation of sourdough changed the mixing behavior of the doughs, resulting in a significant decrease in water absorption ( $P < 0.05$ ) relative to the control. This was not the case for chemically acidified dough (LA), which had a water absorption value similar to that of the control. The dough development time of the five doughs examined differed only slightly; addition of sourdough resulted in marginally longer development times. Larger differences were observed for other parameters. The addition of sourdough prepared from any of the three starter cultures significantly reduced the stability of the dough ( $P < 0.05$ ) relative to both the control and the lactic acid dough (LA) (Table II). Also, large differences were found for the degree of softening; addition of sourdough or acid significantly increased the degree of softening after 10 and 20 min ( $P < 0.05$ ). The biologically acidified doughs (sourdoughs) showed a significantly greater degree of softening than the chemically acidified dough after 10 and 20 min ( $P < 0.05$ ) in all but one case. This indicated major changes in the structure of the sourdoughs that were attributable to more than the addition of acids. Hosoney (1994) reported that 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. Other studies using empirical rheological measurements (Maher Galal et al 1978) also found that the addition of organic acids substantially decreased mixing time and weakened wheat dough. For rye sourdoughs, Wood et al (1975) acknowledged the considerable complexity of the system where microorganisms do considerably more to influence the rheological properties of the dough than simply decreasing the pH value. This is in keeping with the findings of the current wheat dough study where differences were evident between the farinograms of the biologically and chemically acidified dough systems.



**Fig. 5.** Crumb hardness values of loaves baked from wheat dough formulations during a 74-hr storage period: 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 two replicates.

### Extensigraph

The flour used for this study had a mean energy value (area under the extensigraph curve) of 177 cm<sup>2</sup> ( $\pm 12$  cm<sup>2</sup>) and was therefore characterized as being of good quality (Weipert 1993). The flour was within the normal range for the ratio of resistance to extensibility but with a tendency to be a little short as evidenced by a value of 4.9 (Weipert 1993). The effects of sourdough or acid addition on extensibility ( $E$ ) and resistance to extension at 5 cm ( $R_{5cm}$ ) of doughs are shown in Figs. 1 and 2, respectively. The addition of lactic acid or sourdough from a mixed-strain starter culture (MS) significantly reduced  $E$  relative to the control and the single-strain sourdoughs at all three test times ( $P < 0.05$ ). The significant reduction in  $E$  due to the addition of lactic acid is in agreement with Tsen (1966). The only characteristic difference visible from Fig. 2 is the distinctly higher  $R_{5cm}$  value yielded by the chemically acidified dough (LA). This value was also significantly different ( $P < 0.05$ ) from all the other values at all time intervals.

### Fundamental Rheometry

Dynamic oscillatory measurements examined the effects of sourdough or lactic acid on the viscoelastic properties of the dough. The results of these measurements are presented in Figs. 3 and 4. All doughs showed the same trend for phase angle ( $\delta$ ) and absolute value of the complex modulus ( $|G^*|$ ) over the range of frequencies measured. When the frequency increased from 0.1 to 10 Hz, the phase angle values first decreased toward a minimum value before increasing once again. The addition of sourdough or acid did not change this behavior over increasing frequencies. Both the control dough and the chemically acidified dough resulted in the lowest phase angles at all frequencies, and there were no significant differences between these two across all frequencies (Fig. 3). However, the addition of sourdough from any one of the three starter cultures (SS1, SS2, MS) did increase the phase angle values relative to the control or the chemically acidified dough significantly across all frequencies ( $P < 0.05$ ). The phase angle ranges from 0° (ideally elastic material, Hookean solid) to 90° (ideally viscous material, Newtonian liquid). For all viscoelastic materials, the phase angle is between 0° and 90°; the lower the values, the more elastic the material. Therefore, the addition of sourdough reduced the elasticity of the dough in contrast to the chemical acidification, which did not have the same effect.

The absolute value of the complex modulus ( $|G^*|$ ) increased with increasing frequency, and the shape of the curves for all doughs tested was similar (Fig. 4). These data show that the control dough had the highest  $|G^*|$  value over the whole frequency range, indicating that the addition of lactic acid or sourdough reduced  $|G^*|$ . Higher values for  $|G^*|$  indicated that the dough was firmer. There was no significant difference between the  $|G^*|$  values for the control and LA over all frequencies, while the values for the control were significantly greater than those obtained from sourdough over all frequencies ( $P < 0.05$ ). The same was true for the LA, which yielded greater  $|G^*|$  values than the sourdoughs. This relationship was significant in all cases ( $P < 0.05$ ), except at the four higher frequencies, where SS1 showed no significant difference from LA. An increase in phase angle and decrease in  $|G^*|$  due to the addition of sourdough indicated that the dough was less elastic and became simultaneously less firm at the low rate of strain applied (0.1%). No significant differences were found between the  $|G^*|$  values obtained from the sourdoughs across all frequencies, thus indicating that the nature of the starter culture did not have a significant effect on the absolute value of the complex modulus.

It seems logical to assume that chemical acidification per se was not directly comparable with biological acidification within the constraints of this study. This is contradictory to the findings of other authors (Maher Galal et al 1978; Wehrle et al 1997), who highlighted the significant impact of acids on the rheological properties of wheat dough. However, the findings of these authors cannot be

compared directly with those of the current study because the pH value ranges were 3.7–4.2 (Maher Galal et al 1978) and 4.0–5.0 (Wehrle et al 1997). These values are quite dissimilar to the less acidic pH values (5.3–5.4) with the incorporation of 20% sourdough in the white wheat flour system reported here, and may thus preclude direct comparisons. Also, the chemically acidified dough employed here was, unlike its biologically acidified counterparts, devoid of a corresponding 20-hr fermentation period. This, in essence, means that the pH profile of the environment to which the dough constituents were exposed was different for both the biologically and chemically acidified regimes. It can therefore be assumed that the time frame during which enzyme activity could affect the dough constituents was shorter for the chemically acidified dough. This hypothesis supports that recently put forward by Thiele et al (2002), who reported that, with regard to the levels of amino acids in wheat dough, the most important governing factors were dough pH, fermentation time, and the consumption of amino acids by the fermentative microflora.

Several authors have researched the effects of dough moisture content on dynamic viscoelastic behavior of wheat dough. Hibberd (1970) proposed that water molecules in high-moisture doughs behave as inert fillers and that when water content is increased, both the elastic and the viscous moduli decrease. From this, it may be deduced that  $|G^*|$  also decreases with increasing water content. Contrary to this however, the findings of the current study show that although there was a lesser amount of water in all three biologically acidified doughs, there was a decrease in  $|G^*|$ . Therefore, the different rheological response of these doughs must be attributed to a factor other than water content. Hibberd (1970) also indicated that the loss tangent ( $\tan \delta$ ) (and therefore also  $\delta$ ) is independent of water content for a given flour. This also supports the hypothesis that the significant impact of biological acidification must be attributed to other structural changes within the dough.

### Acidification Properties

Data on acidification (pH and total titratable acidity) of fermented sour and bread doughs and bread were compared (Table III). After 20 hr of fermentation at 30°C, the sourdough pH values were 4.0–4.3. These values were within the upper range of values reported by Collar et al (1994a) and Corsetti et al (2000), who have reported sourdough with pH values as low as 3.7 and 3.9, respectively.

It is evident that the standard deviation for both the pH and TTA values reported for the mixed strain starter culture are greater than those of the single strain starter cultures. This may be attributable to the fact that the mixed strain starter culture is composed of living cells that are intolerant to frozen storage conditions and the product thus has a five-week shelf life under chilled conditions, during which time the number of viable cells may diminish. The variable age of the mixed strain starter culture may therefore have introduced variability in the sourdough given that, in adhering to the experimental design, it was not always possible to use the starter culture at the same point in its shelf life. In direct contrast to this situation, the single strain starter cultures had an 18-month shelf life in frozen storage and there was less scope, therefore, for variability in terms of cell viability.

The bread from the traditional mixed strain starter culture had a higher pH and lower total titratable acidity than the single strain starter cultures or the chemically acidified dough. There was however no significant difference between the pH and total titratable acidity values for the sourdough or the chemically acidified breads (Table III). As expected, the unsoured control dough and bread had the lowest acid value, in good agreement with published results (Collar et al 1994a; Corsetti et al 2000). It is important to note that the pH values of the sourdough breads reported here (Table III) are less acidic than those reported in the literature (Collar et al 1994a; Corsetti et al 2000). This may be attributed to the less acidic nature of the sourdough incorporated and is deemed suitable given

that there is not a strong Irish tradition of consumption of sour baked goods (Stear 1990; Cauvain 1998b).

### Rheofermentometer Test

It has been documented by Hammes and Gänzle (1998) that in sourdough systems, yeasts and lactic acid bacteria form carbon dioxide, and the contribution of each group to the overall gas volume differs with the type of starter and dough technology applied. These authors have however noted that, gas formation by the sourdough microflora is only of minor importance if baker's yeast is additionally applied in dough preparation, as was the case in the current study where dried yeast was applied at a rate of 1.5% (fwb). The development and gas retention characteristics of the dough are presented in Table IV. With regard to the maximum dough development height, the control and the mixed strain sourdough yielded significantly greater values than did the single strain sourdoughs ( $P < 0.05$ ). It is important to note, however, that these values might be of little relevance to the expected outcome of practical baking as the time of maximum height for the dough development curve was, in all cases, greater than the 80-min time frame used for the proofing process. No significant differences were found between all five treatments for gaseous release maximum height, appearance time of dough porosity, volume of carbon dioxide produced, lost or retained or gas retention coefficient (data not shown). However, the time at which the gaseous release curve achieved maximum height was much sooner for all three sourdoughs (67–96 min) than it was for the control (114 min) or the lactic acid dough (117 min). This was significant for both SS1 and MS ( $P < 0.05$ ). Unlike the amount of time taken to achieve maximum height for the dough development curve, the time taken to achieve maximum height for the sourdough gaseous release curves was close to the 80-min time frame used for the proofing process.

### Baking Test

Some important results obtained from the baking tests are given in Table V. However, for all bread quality parameters measured, there were no significant differences between the five treatments employed, neither were there any significant interactions between the treatment effect and the day effect ( $P < 0.05$ ). The absence of statistically significant differences between treatments may be attributable to the relatively high coefficient of variation for loaf specific volume, given that loaf specific volume is a primary quality characteristic that can affect many other bread quality characteristics such as textural parameters (Maleki et al 1980). To determine the coefficient of variation, the pooled sample variance was calculated by first taking every treatment and every day as separate samples and then pooling the variances. The pooled sample standard deviation was calculated from this pooled variance. The pooled sample standard deviation was divided by the mean specific volume value to estimate the coefficient of variation (4.8%). A similar coefficient of variation (4.5%) was obtained if separate coefficients of variation were calculated for each day and treatment before being averaged. These values are even lower than those reported by Herendi (1981), who found a coefficient of variation of 5.7% for the volume of loaves prepared during pan bread baking tests. Nevertheless, such values are high in comparison with other analytical methods and therefore may explain why no significant differences were found when the data was subjected to the statistical rigor of split-plot design analysis. However, there were a number of noteworthy trends observed.

Upon the addition of sourdough prepared from any of the three starter cultures, there was an increase in loaf specific volume relative to the control (Table V). This was in distinct contrast to the result obtained upon the addition of lactic acid, which did not show the same improvement in specific volume. This observation is in keeping with previously published work where the addition of sourdough increased loaf volume (Collar et al 1994b; Hammes and Gänzle 1998; Corsetti et al 2000). This trend may be attributed to the findings

obtained from the fundamental rheological tests where the addition of sourdough yielded a less elastic and less firm dough. It may be hypothesized that physicochemical changes in the protein network from the addition of sourdough may have facilitated greater expansion upon proving due to the altogether softer, more extensible nature of the dough.

Increased loaf specific volume values for the sourdough breads were reflected in decreased mean crumb hardness values as measured using texture profile analysis (Fig. 5). Bread profiles generated from digital image analysis showed that the sourdough breads had a greater number of smaller cells, and therefore a greater total number of cells, in the field of vision than the control or the chemically acidified breads (Table V). Again, this is indicative of the effect of sourdough in the production of superior quality breads, as it is generally acknowledged that, for this type of bread, holes of relatively small size ( $\approx 1$  or 2 mm) are required in bakery products, while large voids or irregular crumb distributions are undesirable (Cauvain 1998a).

### Structural Changes Caused by Sourdough

The results of both the rheological and the baking tests show that the incorporation of biologically acidified material produced changes that were different from those seen with chemical acidification. Such changes may be attributed to a number of intrinsically related factors, including variations in the rate or amount of acid produced. The specific properties of the microorganisms present must also be considered.

With regard to gas production in sourdoughs, Hammes and Gänzle (1998) have noted that the contribution of yeasts and lactic acid bacteria to the overall gas volume differs with the type of starter and the dough technology applied. These authors have also noted that gas formation by the sourdough microflora is only of minor importance if baker's yeast is added in dough preparation. It may be assumed, from the results obtained from the current study, that the amount of gas produced by the sourdough organisms does not contribute remarkably to the increase in loaf specific volume. This becomes evident from the fact that there is no distinct difference between the three sourdough bread types with regard to volume, despite the fact that *L. plantarum* is facultatively heterofermentative but typically homofermentative. In contrast to this, *L. brevis* is obligately heterofermentative, and the mixed strain starter culture contained both yeast and an obligately heterofermentative strain in the form of *L. pontis* (Hammes and Vogel 1995). If any sourdough organism contributed remarkably to the bread volume through the amount of gas produced, the volume of that produced using *L. plantarum* should be lower. It may thus be argued that it was the gas retention and not the gas production properties of the dough that was influenced by the addition of sourdough.

Changes in the absolute pH value of the dough system influence its constituent structural components such as gluten. This hypothesis, which has been well documented (Tsen 1966; Maher Galal et al 1978; Wehrle et al 1997; Takeda et al 2001), is supported by the findings of the current study where the results of dough rheology measurements indicated differences between the nonacidified and chemically or biologically acidified doughs. This impact of absolute pH must not, however, be the only reason for changes in the nature of the dough, and this becomes obvious in several instances. The chemically acidified dough is significantly different from the biologically acidified doughs with regard to resistance as determined using the extensigraph, although all have the same pH values. This is true despite the fact that the extensigraph results are not a good reflection of the baking results, as becomes obvious in the lack of difference between the nonacidified control and the biologically acidified doughs with regard to resistance. Also, results obtained from both fundamental rheological tests and farinograms illustrate distinct differences between biologically acidified and chemically acidified doughs but not between the chemically acidified and the nonacidified control dough.

The pH profile may affect the time frame during which the acid influences the constituent ingredients of the dough. The changing pH values during the 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. Thus, the activity of the proteolytic and amylolytic enzymes 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. These enzymes, which play a significant role in terms of their impact on dough constituents, achieve optimum activity at pH 4–5 for the proteolytic and pH 3.6–6.2 for the amylolytic enzymes (Belitz and Grosch 1992). Other enzymes that might affect the structural components of the dough, the activity of which is pH dependent, include lipooxygenases, peroxidases, catalases, and polyphenol oxidases (Belitz and Grosch 1992). Glutathione dehydrogenase may also be worthy of consideration, given that the flour was treated with ascorbic acid (Belitz and Grosch 1992). It is hypothesized that the activity of some or all of these enzymes in the biologically acidified system may have led to structural changes in the dough. This is reflected by the results obtained from the fundamental rheological tests, baking tests, and farinograms where there was a clear divergence evident between the biologically and chemically acidified regimes. The results are also in keeping with the findings of Corsetti et al (2000), who reported that even limited proteolytic degradation of wheat proteins affects the physical properties of gluten, which in turn can have a major effect on bread firmness and staling. Changes in the nature of a sourdough system have also been attributed to the specific proteolytic activity of sourdough bacteria (Spicher and Nierle 1988; Gobbetti et al 1996). However, a recent study by Thiele et al (2002) has indicated that changes in the amino acid profile of the sourdough are caused by the increased activity of flour and not microbial enzymes, as was illustrated by the use of a sterile acidified control subjected to a fermentation period.

## CONCLUSIONS

The addition of sourdough prepared either from a single strain starter culture or a mixed strain starter culture had a significant impact on the rheological properties of wheat flour dough. These changes were reflected in the test baking data. This was in distinct contrast to the lack of effect observed upon chemical acidification of the dough to a comparable pH without the use of a fermentation period. There was no unique trend evident in terms of differences between the effects of the single strain or multiple strain starter cultures with regard to the rheological and baking parameters measured. Generally, the incorporation of biologically acidified material effected distinct changes in the nature of the dough system.

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