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Comparing fermentation gas production between wheat and apple sourdough starters using the Risograph

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ABSTRACT

Production of bread depends on appropriate flour quality to hold fermentation gas, primarily CO_2, and a means of gas production. In typical bread, yeast (*Saccharomyces* sp.) provides the gas during fermentation. This yeast is usually a commercially added monoculture. In sourdough bread production, the fermentation gas is provided by yeast and Lactobacilli that are derived from natural sources and differ greatly depending on which sourdough culture was used. In this study, sourdough cultures derived from wheat, the conventional source of sourdough starter, and apple, an alternative source of non-cereal yeasts were tested for their gas production over time with a Risograph fermentation gas measuring device. Sourdough starter, levain, and bread dough derived from the apple culture significantly out-performed the wheat-derived cultures in CO_2 gas production at every stage of culture elaboration and fermentation. The apple-derived culture produced about twice the fermentation gas of the wheat-derived culture in the bread dough. The results suggested that different sources of wild yeast and Lactobacilli could be used to manipulate the rate, timing and total gas production in sourdough bread-making to modify production parameters and the bread's end-use quality.

1. Introduction

Wheat is unique among cereal grains because wheat flour alone has the ability to form a dough that exhibits the rheological properties required for the production of leavened bread and for the wider diversity of foods that have been developed to utilize these functional attributes. These include a variety of bread products, cookies and crackers, steamed breads, and Asian noodles. Gluten, however, is not enough by itself in a bread-making system. In order to successfully bake a leavened product, leavening gas has to be present as well. Therefore, both the gas production and the ability of gluten to retain leavening gasses are critical in end-use functionality, especially product volume and texture.

Without the presence of leavening gas, any product made from dough would be dense and very hard in texture. In general, leavening gas derives from three main sources: (1) air, typically from a protein foam that has had air whipped into it, as is the case of sponge cakes, (2) carbon dioxide derived from chemical leavening systems in which leavening acids react with bicarbonates, as is the case with cookies and crackers, and (3) carbon dioxide produced by yeast during fermentation, as is the case for most bread.

Yeast-leavened dough systems are varied. Straight-dough, sponge and dough, and sourdough are three predominant systems in which fermentation is used to produce the gas needed to affect volume, texture and to an extent flavor in bread. Yeast added to a dough formulation in commercial

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applications is typically a single cultivated species that produces predictable amounts of CO_2 during fermentation. This system works well in high-throughput facilities where consistency is the goal.

Sourdough systems are more complicated and can be unpredictable in the timing of CO_2 release and the amount released. However, the complexity of the system lends itself to finding more utility in sourdough fermentation than can be found in "monoculture" yeast systems. There has been increasing interest in sourdough systems in recent years due to some research that indicates that sourdough can mitigate the impact of gliadin and glutenin proteins on individuals suffering from celiac disease through microbial cleaving of the proline-rich domains of the gliadins and glutenins contributing to the disease (DeAngelis et al., 2006).

Sourdough systems incorporate more microorganisms than just yeast (Sadeghi, 2008). The general sourdough process involves a culture of yeast and lactic acid bacteria that are cultivated through several generations to achieve a stable system and then used to ferment doughs. At each generation, some of the previous dough is retained for use as a starter for the next dough that is mixed and to maintain viability of the culture. Consecutive microbial reinoculation is referred to as backslopping (Häggman & Salovaara, 2008).

Yeast and lactic acid bacteria are the two main types of microorganisms that make up the flora present in the sourdough process. Because every microorganism needs a specific environment with favorable conditions for reproduction, the type and quality of each will be affected by the sourdough's characteristics including hydration, ingredients (salt content being particularly influential), temperature, acidity, and flour composition. Yeast (*Saccharomyces cerevisiae*, and others) used in sourdough is typically of a "wild" or uncultivated type, derived from nature. Generally, wild yeast is more resistant to acidity compared to commercial yeast, leaving it better adapted to the sourdough process.

The presence of lactic acid bacteria (*Lactobacillus* sp.) alters the dough pH, titratable acidity, and the ratio of lactic and acetic acids in the fermentation system (Hammes and Ganzle, 1988). The changes in the dough contributed by the lactic acid bacteria alter the dough handling properties and texture (Clark, Schobr, Dockery, O'Sullivan, & Arendt, 2004), the flavor of the bread (Gobbetti, DeAngelis, Corsetti, & DiCango, 2005; Ur-Rehman, Paterson, & Piggott, 2006), and the environment in which the yeast grows and produces leavening gas that contributes to loaf volume (Corsetti et al., 1998). Lactic acid bacteria originate from flour, dough ingredients, or from the environment (Gobbetti et al., 2005).

The array of species of yeast and lactic acid bacteria that are available to serve as sourdough starter is vast. The initial source of the starter culture, given the preference these microorganisms display, can have a large impact on how the organisms in the starter function in a dough system. Most sourdough cultures for bread production derive from sources associated with cereals, grain, or flour. However, other sources of yeast and lactic acid bacteria from other plant sources have not been well explored. Yeast and lactic acid bacteria of different strains from those associated with cereals are present on apples, grapes, peaches and other high-sugar fruits. Previous research has shown that the sourdough bread produced with cultures derived from fruit sources is less sour than that from cereal flour-derived starters (Cheng, 2002).

This study described the preparation procedures of sourdough starters derived from apple and from cereal sources and compared their fermentation power. The fermentation power was determined through the measurement of the CO_2 produced from dough fermentation by a specialized instrument that measures gas production from chemical or microbiological sources.

2. Materials and methods

2.1. Materials

For sourdough production, three flour types were used: hard red winter wheat flour (HRW, 0.53% ash, 11.78% protein and 13.9% moisture); dark rye flour (1.45% ash, 8.02% protein and 11.06% moisture); and fine stone-ground whole wheat flour (1.97% ash, 14.47% protein and 11.05% moisture). HRW was obtained from Pendleton Flour Mills, Pendleton, OR. Dark rye flour and fine stone-ground flour were obtained from Bob's Red Mill, Milwaukie, OR. Cameo apples were obtained from Washington State.

2.2. Preparation of sourdoughs

Sourdough production followed the order: Preferment→Culture→Sour Starter→Levain→Bread Dough. HRW flour was used for the sourdough production from Culture through Bread Dough. The dark rye flour and fine stone ground whole wheat flour were used to prepare wheat preferment, and the apple was coupled with sugar to prepare the apple preferment. Preferment is designed to incubate the natural yeasts and lactic acid bacteria from the flour and apple sources. Each stage of the preparation of the sourdough is iterated below.

2.2.1. Preparation of the preferments

Wheat preferment was prepared with the method of the San Francisco Baking Institute method (Suas, 2009). Fine stone-ground flour was combined with dark rye flour (200 g each) and 200 g water and incubated at 28 °C for 24 h.

Apple preferment was prepared with the method of Cheng (2002). Apples were rinsed with water, wiped to remove water on surface, and cubed. Sugar, cubed apple and water (200 g, 500 g and 200 g, respectively) were pureed and incubated at 28 °C for 96 h.

2.2.2. Preparation of the cultures

The first wheat culture was prepared by mixing 300 g wheat preferment with 300 g HRW flour and 300 g water. The culture was held at 28 °C in a proof box and then remixed twice a day for seven subsequent mixes (Suas, 2009). Each subsequent mix used 300 g of the previous batch of culture, with 300 g each of HRW flour and water.

The first apple culture was prepared by mixing 300 g apple preferment with 300 g HRW flour and 150 g water, held at 28 °C in a proof box and remixed twice a day. The subsequent seven mixes were made using 300 g of the previous culture, with 300 g HRW flour and 300 g apple puree (225 g water and 75 g cubed apple).

2.2.3. *Preparation of the starters*

The first wheat starter was prepared by mixing 100 g wheat culture, 200 g water and 400 g HRW flour. The starter was held at 28 °C in a proof box for 24 h between mixes. The subsequent seven mixes were made by mixing 25% of the previous culture with 400 g HRW flour and 200 g water.

The first apple starter was prepared by mixing 100 g of the apple culture, 50 g apple puree (12.5 g apple, 37.5 g water), 150 g water and 400 g HRW flour. The subsequent seven mixes were made by mixing 100 g of the previous starter with 400 g HRW flour, 150 g water and 50 g apple puree. Each starter was held at 28 °C for 24 h between mixes.

2.2.4. *Preparation of the levains and bread doughs*

Levain is the final bread leavening agent, and produces breads with rich aroma, pleasant structure and excellent keeping properties. Sourdough breadmaking contains two steps of mixing: levain and bread dough. For levain production, 25 g starter (apple or wheat) was mixed with 95 g HRW, 5 g rye flour and 50 g tap water and incubated at 28 °C for 12–24 h to form levain. Then, 50 g levain was mixed with all ingredients including 100 g HRW, 74 g tap water, 0.1 g instant yeast, and 2.4 g salt and fermented at 28 °C for 2 h to form bread dough (Suas, 2009).

2.3. *Measurement of fermentation gas production using the Risograph*

Gas evolution at various stages of the sourdough production was measured with the Risograph (National Manufacturing Co., Lincoln, NE), following the general procedures of AACCI Approved Method 89-01 (AACC International, 2012). The Risograph is unique in its approach to measuring the evolution of leavening gas (Häggman & Salovaara, 2008; Walker, Rattin, Faubion, & Mense, 2009). Samples of dough are incubated in jars in a temperature-controlled water bath. As gas evolves, it is measured as back pressure and converted into volume via the gas law equation $PV=nRT$. After the measurement of pressure, the gas is released returning the cell to atmospheric pressure. The elimination of back-pressure allows the yeast to fully express gas during the entire fermentation process. The data obtained are the rate of gas evolution, production of gas during any time interval and cumulative gas production.

2.4. *Statistical analysis*

All measurements were conducted in triplicate and statistical analyses were done using SAS v9.3 (SAS Institute, Cary, NC). LSD was generated through SAS Proc GLM with LSD selected as the method stipulated for mean comparisons. The number of measurements for each paired comparison was 6 (2 preparations × 3 measurements).

3. Results and discussion

3.1. *Comparison of CO_2 gas production during culture elaboration*

Differences in the amount of gas evolved from yeast and Lactobacilli derived from wheat and apple sources were evident from the very first step of culture creation. At the first culture stage, the wheat-based culture produced more CO_2 than the apple-based culture in the first few hours of fermentation, as measured with the Risograph. However, the apple-based culture produced more gas after 5 h of fermentation and maintained higher production of gas after that (Fig. 1A and B). As backslopping occurred, wheat-based cultures produced very little gas in the 2nd to 4th culture stage (Fig. 1B). In subsequent culture stages, 5 through 8, both cultures evolved gas at relatively high rates in the first 11 h. Wheat-based cultures had a steeper decline in CO_2 production after the peak gas production rate than did the apple-based cultures. Thus, the total amount of gas produced was greater at all culture stages for the apple-based cultures than the wheat-based cultures.

At the end of eight culture stages, the cultures were essentially stabilized in terms of their respective gas production (Fig. 1A and B), indicating that the microbiological distribution of species had also stabilized. At this point the culture was used to initiate a starter, and CO_2 production was monitored, again with the Risograph.

Sourdough microflora from wheat flour generally contain a complex mixture of yeasts (mainly *S. cerevisiae*) and hetero- and

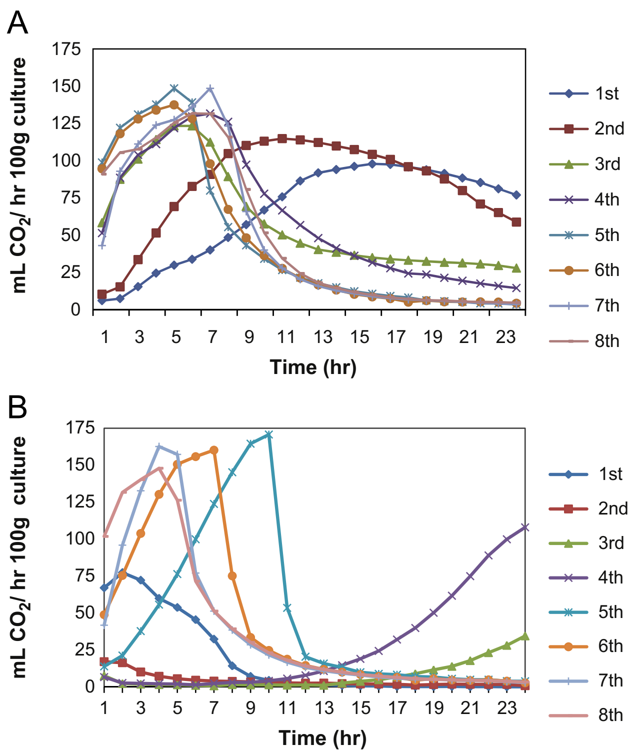

Fig. 1 – CO_2 production rate (mL/h/100 g culture) for cultures derived from apple (A) and wheat (B). Number of each line is the number of backslops for each culture.

homo-fermentative LAB. A large number of *Lactobacillus* species have been isolated including *Lactobacillus Sanfranciscensis, Lb. points, Lb. brevis,* and *Lb. plantarum strains* (Ottogali, Galli, & Foschino, 1996; Gobbetti, 1998; De vuyst et al., 2002). These yeast and LAB strains contribute to the dough fermentation and gas production. However, there is little scientific report of preparing sourdough culture and starters from apple source. In one study (Nyanga et al., 2007), yeasts, yeast-like fungi, and lactic acid bacteria present on the unripe, ripe and dried fruits, and in the fermented *masau* fruits collected from Muzarabani district in Zimbabwe were isolated and identified using physiological and molecular methods. Among species that were identified, *S. cerevisiae*, *Issatchenkia orientalis*, *Pichia fabianii* and *Aureobasidium pullulans* were predominant. The authors pointed out that *S. cerevisiae*, *I. orientalis*, and *P. fabianii* are fermentative yeasts and these might be used in the development of starter cultures to produce better quality fermented products from *masau* fruit. Of the lactic acid bacteria (LAB) that were identified, *Lactobacillus agilis* and *L. plantarum* were the main strains. Other species identified included *L. bifermentans*, *L. minor*, *L. divergens*, *L. confusus*, *L. hilgardii*, *L. fructosus*, *L. fermentum* and *Streptococcus* spp. Some of these LAB could also potentially be used in a mixed-starter culture with yeast strains and might contribute positively in the production of fermented products. Presumably, the apple may have similar yeast and LAB strains to masau fruits and exhibits more profound gassing power than the wheat flour culture.

3.2. Comparison of CO_2 gas production during backslopping of starters

As can be seen in Fig. 2, the first several iterations of starter stages had high initial gas production rates. When the apple culture was used to create starter, high initial gas production rates were attained between hours 6 and 7 and maintained until about 13 h, at which point gas production rates waned (Fig. 2A). This pattern of gas production rate was observed in 1st to 5th starter stages. At the 6th stage and beyond, the highest gas production rates decreased somewhat, but still showed peak gassing rates occurring between 5 and 13 h. Again, as in the culture stage, the gas production rate stabilized in stages 6–10, indicating that the microbiological flora had also stabilized.

Wheat starter showed a similar pattern (Fig. 2B), but with peak gas production rates decreasing more rapidly as the starter stages progressed. For the wheat starter, the highest gas production rates occurred only in stages one, two and three. The final, stable pattern of gas production was achieved by the 5th stage. For the wheat starter, the CO_2 peak production rate occurred between 8 and 15 h, as opposed to the apple, where the peak rates (in stages 6–10) occurred between 6 and 13 h. Additionally, the peak rate of production was less in stages 5–10 for the wheat-derived starter than for the apple-derived starter.

3.3. Comparison of fermentation power of levains

When measured over 24 h, levain gas production rate (mL/min/100 g levain) showed that maximum gas production rates occurred at around 6 h for levain made with the apple starter (2.31 mL/min) and at 10 h for levain made from the wheat starter (1.15 mL/min) (Fig. 3A, and Table 1). Gas rates were measured in 1-min increments, but data are only presented here at half-hour intervals for the first 6 h and 1 h intervals subsequently. The maximum gas production rate for the apple levain was twice that for that of the wheat levain. Rates decreased steadily after the time at which maximum peak gas production rate occurred (Fig. 3A and Table 1). Although the apple levain had greater gas production rate and an earlier peak time, the wheat levain gas production rates had less rapid increase and decrease in production rate and a less steep gas rate curve resulted (Fig. 3A and Table 1).

The apple levain gas production rates were significantly greater (α=0.05, Table 1) than those of the wheat levain until hours 11 and 12, during which times wheat and apple levains were not different. Between fermentation times of 13 and 20 h, the wheat levain had a significantly greater gas production rate than did the apple levain. At hours beyond 21, both rates had decreased to the point of having no significant difference. For rapid gas production rate, the apple levain was significantly greater than the wheat levain overall (Table 1).

When translated into total gas produced, the influence of the greater gas production rate of the apple levain is apparent (Fig. 3B). Total gas production fall on sigmoid lines, as shown in Fig. 3B, as calculated from the major points, but intermediate values also fall on the calculated curves. Levain produced from apple had significantly (α=0.05, data not shown) greater total gas production than did the wheat levain for every time value measured. Generally, the apple levain produced about twice the total

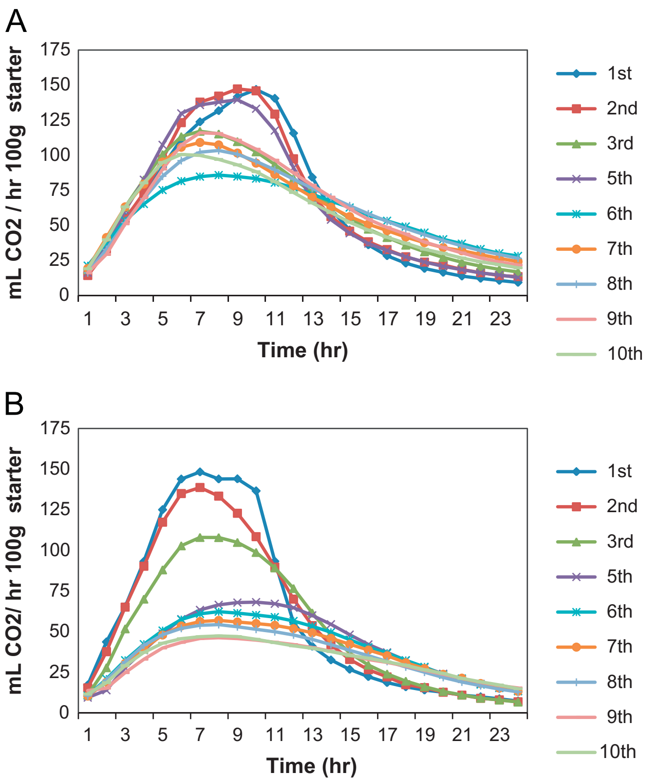

Fig. 2 – CO_2 production rate (mL/h/100 g starter) for starters derived from apple (A) and wheat (B). Number of each line is the number of backslops for each starter.

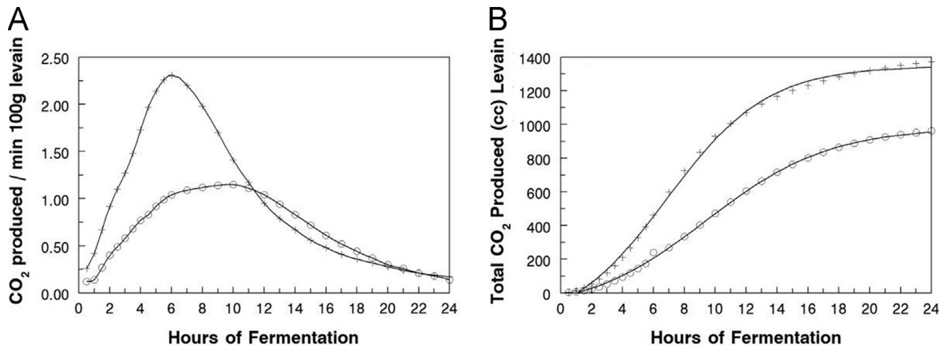

Fig. 3 – CO_2 production in levain. Gas production rate (cm^3/min/100 g levain) in A; total gas production (cm^3/100 g levain) in B for apple (+) and for wheat (o).

Table 1 – Rate of CO_2 gas production from levain comparing apple and wheat cultures.

Time (h)	Levain gas rate from source[a]		LSD	p-value[b]
	Apple	Wheat		
0.5	0.26	0.12	0.057	0.003
1	0.42	0.14	0.052	0.0001
1.5	0.67	0.27	0.069	<0.0001
2	0.92	0.40	0.081	<0.0001
2.5	1.10	0.49	0.091	<0.0001
3	1.27	0.58	0.091	<0.0001
3.5	1.48	0.68	0.102	<0.0001
4	1.73	0.77	0.107	<0.0001
4.5	1.97	0.83	0.109	<0.0001
5	2.14	0.92	0.049	<0.0001
5.5	2.26	0.99	0.107	<0.0001
6	2.31	1.04	0.085	<0.0001
7	2.20	1.09	0.110	<0.0001
8	1.98	1.12	0.101	<0.0001
9	1.70	1.14	0.082	<0.0001
10	1.41	1.15	0.070	0.0005
11	1.17	1.11	0.116	0.2254
12	0.95	1.04	0.093	0.0549
13	0.79	0.94	0.079	0.0067
14	0.67	0.83	0.065	0.0026
15	0.56	0.72	0.058	0.0015
16	0.48	0.61	0.043	0.0013
17	0.41	0.52	0.041	0.0018
18	0.36	0.44	0.050	0.0085
19	0.32	0.37	0.041	0.0285
20	0.28	0.30	0.016	0.0257
21	0.24	0.26	0.035	0.1347
22	0.21	0.21	0.026	1.0000
23	0.19	0.18	0.029	0.2746
24	0.17	0.14	0.025	0.0390

[a] Mean of six measurements (2 preparations × 3 measurements); mL CO_2/min from 100 g levain.
[b] Differences in gas production are significant when p-values are less than 0.05.

gas as did the wheat levain up to about 9 h of fermentation time, and 1.7 times the total gas as produced by the wheat levain up to about 13 h of fermentation time. After that, the fact that the wheat levain had greater production rate later in the fermentation than did the apple levain, reduced the difference between the two levain types to about 40% at 24 h of fermentation time (Fig. 3B).

Although the wheat levain gas production rates were greater than those of the apple levain later in fermentation time, the total amount of gas produced by the apple levain early in the fermentation was much greater than that of the wheat levain and the amount of gas produced by the wheat levain never equaled to that of the apple levain.

In a typical commercial sourdough bread production, the levain is produced by mixing sour starter with flour and water and fermented for 12 h before it is used in the final dough preparation (Suas, 2009). The first 12 h of fermentation time is of much greater practical interest to bakers than periods extending beyond that. Closer examination of the total gas produced in a dough system is therefore of greater practical interest than monitoring gas production in levain after up to 24 h of fermentation.

3.4. *Comparison of CO_2 gas production of bread sourdoughs*

Total gas production was significantly greater for the wheat levain in bread dough ($\alpha = 0.05$, Table 2) for the first 10 min of fermentation. This is a case where statistical difference exists, but is not meaningful in a practical sense. The amount of gas produced is relatively small in both wheat and apple dough systems during the first half hour or so of the fermentation process. For minutes 10–25, total gas production did not differ between the two levain types in dough (Table 2). After 30 min, the apple sourdough produced significantly more total CO_2 than did the wheat sourdough. The total gas production difference between the two sources of CO_2 persisted throughout the rest of the 2-h fermentation (Fig. 4). After 2 h of fermentation, the sourdough fermented by the apple levain produced 130% more total CO_2 than that of the wheat levain (Table 2).

4. Conclusion

The source of the microbiological origins (yeast, *Saccharomyces* sp. and Lactobacilli) of fermentation gas in sourdough bread systems have a great impact on the eventual rate and

Table 2 – Total CO_2 production from sourdough comparing apple and wheat cultures.

Time (min)	Total CO_2 produced in 100 g sourdough from source[a]		LSD	p-value[b]
	Apple	Wheat		
5	1.6	2.5	0.39	0.0096
10	2.9	3.8	0.52	0.0178
15	4.2	4.9	0.72	0.0545
20	5.5	5.7	0.77	0.3190
25	7.0	6.6	0.73	0.1305
30	8.5	7.2	0.65	0.0148
35	10.2	8.1	0.53	0.0033
40	12.2	8.9	0.58	0.0017
45	14.4	9.7	0.60	0.0009
50	16.8	10.6	0.59	0.0005
55	19.6	11.7	0.74	0.0005
60	22.7	12.7	0.64	0.0002
65	26.0	13.8	0.57	0.0001
70	29.7	15.0	0.53	<0.0001
75	33.7	16.4	0.60	<0.0001
80	37.9	17.9	0.60	<0.0001
85	42.3	19.6	0.60	<0.0001
90	47.0	21.3	0.62	<0.0001
95	51.9	23.2	0.53	<0.0001
100	57.0	25.2	0.62	<0.0001
105	62.3	27.2	0.67	<0.0001
110	67.8	29.4	0.72	<0.0001
115	73.5	31.8	0.82	<0.0001
120	79.3	34.3	1.05	<0.0001

[a] Mean of six measurements (2 preparations × 3 measurements); Total mL CO_2/min/100 g dough.
[b] Differences in gas production are significant when p-values are less than 0.05.

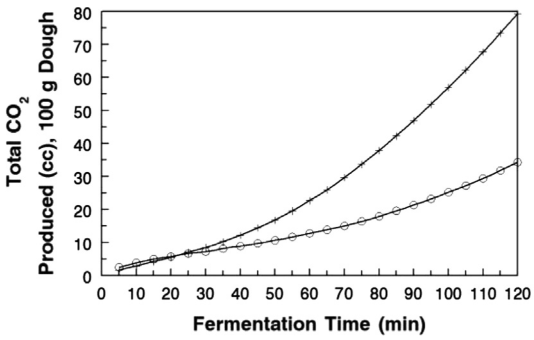

Fig. 4 – Total CO_2 production for sour dough bread dough (cm^3/100 g dough) using starter from apple (+) and from wheat (o).

amount of total gas produced. Careful selection of sources of sourdough starter can have a large effect on the final product. Further, selection of different sources of starter can allow greater control of the timing and amount of gas produced during fermentation.

In this study, two diverse sources of microbial flora were selected. One set was a traditional grain-based culture. The other culture was derived from the yeast and lactobacilli present on apples, not normally used to prepare sourdough starters. Differences in the rate and the timing of gas production were immediately evident in the rate and total amount of gas produced, as measured with the Risograph. The differences between the two cultures persisted through progressive stages of starter and levain production. Apple-based cultures, starter, levain and sourdough all had significantly greater gas production, total and rate, than did the gas production from the wheat-based sourdough cultures. The apple sourdough starter may contain yeast and LAB strains that are not present in flour sourdough starter and provide additional benefits to fermentation and gas production.

Measurement of gas production by Risograph provides a repeatable, accurate measure of gas evolution during fermentation. The results indicate that the potential for manipulation of sourdough systems exists based on the selection of the starter culture. This study was a first observation of the phenomenon and further research should be conducted to explore the differences in yeast and LAB strains between the flour and apple sourdough starters that contribute to large variations in gassing power. Further study is also needed to investigate the effects of different sources of microbial flora on the sourdough bread quality in terms of loaf volume, aroma, texture, crumb grain, crumb color, and crust color.

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