



Measuring Yeast CO₂ Production with the Risograph

- ▶ The Risograph was developed as a device to measure gas production in yeast-leavened doughs.
- ▶ Little information has been published on this device or factors affecting its performance.
- ▶ Tests done with these instruments are repeatable, but the results obtained can depend upon several factors, including the mixer used.

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Wheat flour is widely used in baked products because of its unique properties. In products such as bread (10,15), leavening is fundamental to obtain a product that has the required textural characteristics. Leavening action modifies the cellular structure of the dough by expanding gas cells with CO₂ (4,6,7). Yeast produces gas during fermentation, while the gluten proteins are responsible for the dough's unique capacity to retain the gas produced. This ability to produce and retain gas results in lightly textured products (10,11,15). Therefore, a reliable and convenient method for measuring gas production has long been a goal of many cereal chemists.

While some studies have been conducted with the Risograph, little information is available in the literature about its characteristics. This article reports on its ability to provide standardized measurements on the production rate and the total amount of CO₂ produced per gram of dough and on its ability to observe differences in the re-

sults obtained from two different operators, from two different Risographs, and in the three-pin mixers used.

Methods for Measuring Gas Production

Over the years, several methods have been proposed and developed to measure the gas produced in bread doughs as a result of yeast activity. Gas production was measured volumetrically by several research groups (3,5,12,13,14,17,18) and the procedure was later improved with the use of inversely calibrated burettes and water baths for temperature control (15,16) (Fig. 1).

Once their practicality had been demonstrated (8), the most commonly used methods made use of a pressure gauge on a sealed container. Workers in the pre-computer era compared gas production results from instruments that required periodic as well as endpoint readings and concluded that these methods were not the most suitable since periodic readings were costly in terms of time and effort, while endpoint-only readings could miss rate changes that might reflect upon fermentation chemistry (19). The need for a more practical and sensitive continuous gas release recording device led to the development of a simple piece of equipment, the Gasograph. The instrument continuously measured and graphically recorded gas production directly by volume for up to 12 sample containers (16) (Fig. 2).

The Gasograph was replaced by the Risograph—a pressure-measuring, rather than a direct-volume-measuring device (R-Design Co., Pullman, WA, U.S.A.). It

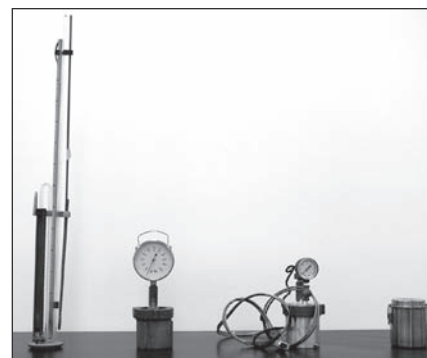


Fig. 1. Historical sequence of gas production measuring equipment, from left to right, mercury manometer, pressure gauge, computerized gauge pressure meter, and Risograph sample container.

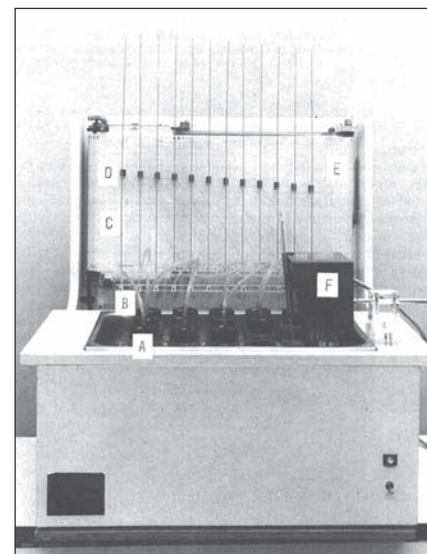


Fig. 2. The Gasograph. Source: Rubenthaler et al., 1980 (16).

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rapidly and accurately determines the milliliters of CO₂ evolved per minute, as well as the cumulative total, using proprietary software to control sampling, data collection, reduction, and display, and to report preparation. Gas production is measured independently in each assembly (container, tube, and port system) through a precise pressure sensor. The gas released is transferred to the measurement chamber, where it is measured as pressure and the values are converted to volume in milliliters at standard temperature and pressure, using the perfect gas laws. A total of 12 samples can be tested at the same time in a given run. Although it is a direct reading and automatic instrument, errors in measuring the release of gas caused by leaks, variation in barometric pressure, variation in temperature (dough and the measuring device), calibration, and standardization of ingredients (flour, yeast, water, etc.) can still occur (9).

The AACC Intl. Method 89-01.01 yeast activity for gas production (1) lists several types of equipment that could be used to measure gas pressure, but it does not provide specific methods. Rather, it attempts to standardize yeast pretreatments and dough formulations. The latter are calculated so that equal weights of dough contain equal weights of yeast solids (0.70 g per 100 g of dough), which allows comparisons of gassing activity among different yeast types (9).

The Risograph and How It Works

The Risograph consists of a chassis that has 12 gas sample container ports, a measuring port, and a vent port. The “measuring container” and lid always remain empty with the lid connected to the measuring port, serving to hold the gas from each sample container in turn, as its pressure is being measured, before being vented to the atmosphere. The sample containers reside in a water bath, in which the temperature should be set (usually at

30°C) and allowed to stabilize for 1 hour before a run starts. Containers and lids should be matched and permanently marked to ensure that the operator always uses the same lid and container on the same port (2) (Fig. 3).

The Risosmart software can simultaneously collect data from two different Risograph instruments (20). The test begins after dough samples are placed into the stainless steel containers, the lids and hoses attached, and the connections made to the specific sequence-numbered chassis port (1 to 12). Each sample container tested is followed separately by a real-time clock, and the plotted results are adjusted for elapsed time for that specific sample, i.e., time shifted for graphical comparisons. The measurement cycle operates as follows: for each container in the sequence, the gas produced is transferred to the calibrated measuring chamber connected to

its specifically numbered port. The pressure is then measured and the gas is vented to the atmosphere through the vent port, which is always open. This sequence, transfer, measurement, and venting, is repeated for the other samples in order, once their respective run starts, and is repeated at the set interval, generally 1 min, until total run time is reached (2).

For each container, gas production is reported in volume, as milliliters of CO₂ released per minute (rate) and as cumulative milliliters of CO₂ released over time (total). Data is automatically plotted as shown in Figures 4 and 5.

When the run is completed (typically after 90 min), the connecting tube is removed from the lid first. This will prevent accidental pressure buildup and container overflow into the open tubing, resulting in valve damage. Once the run is completed, lids and containers are washed, dried, and



Fig. 3. Risograph equipment. This model will accommodate 12 sample containers plus the “M” measuring reference container, though only six containers are shown.

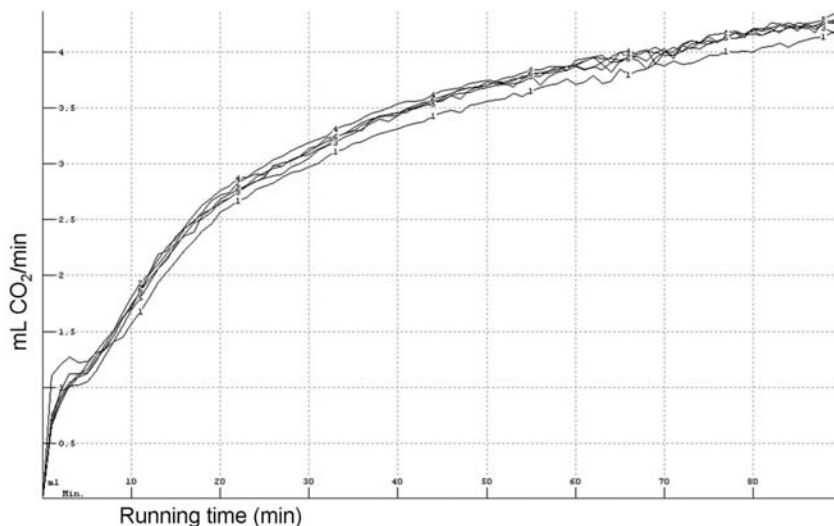


Fig. 4. Sample rate plot, milliliters of CO₂ per minute.

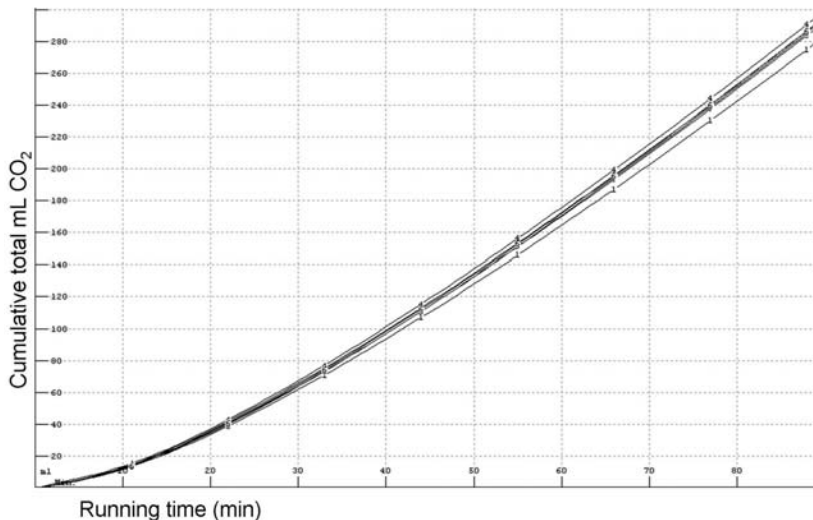


Fig. 5. Sample cumulative plot, total gas released in milliliters of CO₂.

a thin film of silicon lubricant applied to the lid rim “O” ring. Lubricant eases the connection and disconnection of the lid, as well as lengthens the life of the gasket and improves the gas seal. The containers are returned to the water bath to maintain its water level at a constant position.

Mixing Procedure

The bread dough formulation employed was that specified in AACC International Method 89-01.01, yeast activity and gas production (1), using instant active dry yeast (IADY). The doughs were prepared with a 35-g pin mixer, a 100-g Finney Special pin mixer, and a 200-g pin mixer (National Manufacturing Division, TMCO, Lincoln, NE, U.S.A.). The 35-g pin mixer provided one dough of approximately 60 g. This was taken as the standard sample size to be used for all tests. This mixer was used to evaluate six replicate doughs individually mixed. The 100-g pin mixer provided a dough weighing approximately 184 g, which when divided resulted in three samples of 60 g each. A second mix resulted in a total of six samples, which resulted in one experimental run. Finally, the 200-g mixer provided six 60-g dough samples in a single mix. In this manner, the dough weight tested was the same in all cases, so the total normalized gas production in milliliters per gram of dough could be used to compare for differences in mixers (Fig. 6).

Operator and Risograph Effect Using a 35-g Pin Mixer

Two different operators prepared dough samples using the 35-g mixer, tested with the two Risographs, and replicated the experiment on separate days. Each replicate is reported as the average of six containers from the same experimental run (Table I). There were no significant differences between operators or instruments. This is important because, in many quality control situations, the tests may be conducted by different operators or on different instruments (perhaps even at different locations).

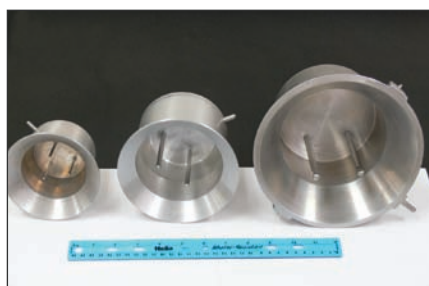


Fig. 6. Mixing bowls, from left to right, 35-g pin mixer, 100-g pin mixer, and 200-g pin mixer.

The same study was conducted with the 100-g pin mixer and each dough was divided into three measuring containers. Significant differences were observed in normalized CO₂ production (milliliters per gram of dough) between operators 1 and 2 (data not shown).

Mixer Differences

In an attempt to identify possible differences arising from the mixer chosen, an experimental series was run using three mixer sizes. The same operator ran both Risographs, again with six sample containers per run. When the 35-g mixer was used, one dough sample was created at a time, one after the other. When the 100-g mixer was used, two doughs were mixed in sequence, and each was divided into three samples. When the 200-g mixer was used, one dough was mixed but divided into six samples.

The normalized total gas production (milliliters per gram of dough) means and standard deviations for the 35-, 100-, and 200-g mixers are shown in Table II.

When using device A, significantly smaller amounts of gas were produced when using the 35-g mixer compared with that when using the 100- and 200-g mixers. Significantly smaller amounts of gas were produced when using the 35- and 100-g mixers in comparison with that when using the 200-g mixer with device B. When all mixers and both devices were compared, the 35-g pin mixer resulted in a significantly smaller amount of gas being produced when compared with that resulting from the 100- and 200-g mixers.

It was hypothesized that such differences may have been caused by the differences in timing of the samples’ placement in the measuring containers. With the 35-g procedure, each sample was mixed individually, removed from the bowl, and weighed. The temperature was then quickly taken, after which it was placed into the sample container, so there was little time available for the yeast to be activated and fermentation to begin. However, when the 100-g mixer was used, three dough samples were obtained from each mix, so it was impossible for the operator to weigh and take the temperatures from all of them at the same time. Thus, at least two samples remained at the bench longer than the individual samples obtained using the 35-g pin mixer. Yeast activation starts as soon as the ingredients are combined and fermentation continues during bench time, so the average total gas release obtained after 1.5 h was higher for the 100-g mixer than for the 35-g pin mixer.

A similar response phenomenon was also observed using the 200-g pin mixer and its “six-samples/mix” doughs. Not only was the amount of CO₂ produced higher on average compared with the amounts from the smaller mixers, it increased from the first sample to the last of six within a single run (data not shown). Clearly, the mixer size had an indirect effect on the results.

IADY starts fermenting the sugar available in the dough as soon as it is combined with the other ingredients, especially with water. In the beginning, a great amount of sugar is available, and the rate of CO₂ pro-

Table I. Normalized gas production in milliliters per gram of dough^a

Risograph	Operator 1	Operator 2
A	4.52 ± 0.51 a	4.67 ± 0.24 a
A	4.68 ± 0.19 a	4.66 ± 0.13 a
A	4.73 ± 0.15 a	4.76 ± 0.13 a
A (average of three replicates)	4.64 ± 0.32 a	4.70 ± 0.17 a
B	4.65 ± 0.14 a	4.75 ± 0.13 a
B	4.79 ± 0.13 a	4.65 ± 0.34 a
B (average of two replicates)	4.72 ± 0.15 a	4.70 ± 0.25 a

^a Obtained for operators 1 and 2 on different days using Risographs A and B with the 35-g pin mixer. Each cell lists the average and standard deviation of six dough samples tested at one time. Comparisons between operators using a given instrument were determined using the GLM and LSD procedures from SAS. The same letters within a row indicate no significant difference ($P \geq 0.05$).

Table II. Normalized gas production in milliliters per gram of dough^a

Mixer	Risograph A	Risograph B	Average
35-g mixer	4.97 ± 0.1 ac	4.91 ± 0.13 ac	4.94 ± 0.11
100-g mixer	5.06 ± 0.09 ac	5.11 ± 0.08 ad	5.09 ± 0.08
200-g mixer	5.24 ± 0.12 ad	5.13 ± 0.11 ad	5.19 ± 0.12
Average	5.09 ± 0.15	5.05 ± 0.15	5.07 ± 0.15

^a Obtained for operator 2 using three mixer sizes and two Risographs. Each reported value is the average and standard deviation of six dough samples. Comparisons between equipment and mixers were determined using the LSD procedure. The same letters within a row (a) indicate no significant difference between the two Risograph instruments ($P \geq 0.05$). Different letters within a column (cd) indicate significant differences among the mixers ($P < 0.05$).

duction per minute proceeds rapidly as the rate curve climbs, reflecting activation of the system. As time passes, less sugar is available in the immediate vicinity of the yeast, and gas production proceeds at a lower rate, flattening the curve (Figs. 4 and 5).

However, in spite of its name “instant,” IADY does take time to activate, and to reach the full gas production rate. It was hypothesized that the additional bench time caused the increasing rate of gas production for the larger mixers because the later samples began data collection at a higher rate of gassing activity.

The hypothesis described above was confirmed when dough samples subjected to different bench times (0, 3, 6, 9, 12, and 15 min) were tested, using the 35-g pin mixer. The normalized gas production in milliliters per gram versus time is shown in Figure 7. There were significant differences in normalized total gas production between 0, 3, 6, and 9–15 resting minutes. Taken together, these studies show that once the yeast is combined with the other ingredients, waiting time prior to placing the sample in the containers affects the amount of gas measured during the test.

The interval between the actual start of testing measurements is crucial for good repeatability, because a “small” delay of 3 min was enough to result in a significant difference in measured gas generation rate and total production in 90 min. Because the 35-g pin mixer procedure did not affect the method’s repeatability when Risographs and operators were compared, it is the recommended mixer size for determining gas production in yeasted dough systems. Mixing with the 35-g pin mixer results in only one sample, allowing the time between mixing and placement of the

sample in the sample container to be both shorter and more consistent.

Conclusions

A standard method was developed and the results were shown to be repeatable between two operators using two Risographs and a 35-g pin mixer. However, when larger mixers are used, the values become less consistent. The variability was found to be caused by the increased time required to process the mixed dough samples before measurement. The 35-g pin single-sample-per-dough mixer is therefore recommended for this type of analysis, if a sample size of 60 g is satisfactory.

Leavening gas production and retention is directly related to the end quality of several yeast-leavened products, mainly bread. The loaf volume and crumb grain is dependent on yeast activity and gas retention in the gluten matrix structure. Accurate and practical instruments such as the Risograph combined with a standardized method are recommended as a daily quality control analysis technique as well as a tool for the identification of yeast activity inhibitors or stimulators, and for the investigation of formula ingredient functionality. Additionally, different mixing methods can be tested as well as different fermentation times. The effect of such factors as fermentation time and temperature can also be tested. Complex analysis, using combinations of different levels of ingredients, may be suitable for product improvements and for new product development.

Areas for future work include studies on the effects of external barometric pressure and temperature. The Risograph has the potential to be used as a general purpose manometric instrument for testing gas production from different sources, includ-

ing applications in chemical leavening products, biofermentations, yeast strain development, interactions of sugar and yeast concentrations, and oxygen consumption studies.

Acknowledgments

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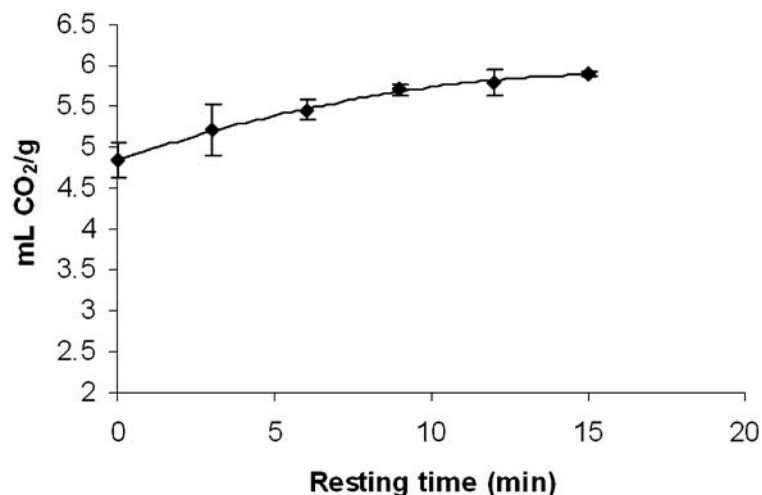


Fig. 7. Normalized gas production obtained using a 35-g mixer with different bench resting times (minutes) before beginning the Risograph run. There were significant differences in total gas production between 0, 3, 6, and 9–15 minute intervals.

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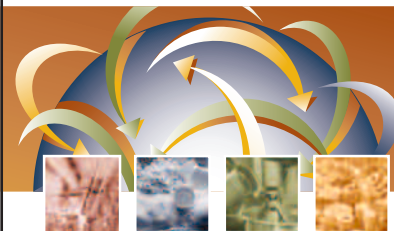


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