

A Rapid Method for the Determination of Yeast Dry Weight Concentration¹

James F. Rice, Timothy R. Sullivan,² and J. Raymond Helbert, *Miller Brewing Company, Milwaukee, WI 53201*

ABSTRACT

A rapid method using membrane filtration and microwave drying was developed for measuring yeast dry weight concentration. It permits the determination of yeast dry weight in about 0.5 hr and has the same precision as a conventional method utilizing centrifugation and hot air drying. The microwave method gives dry weight measurements about 5% greater than those obtained by the conventional method. Correlation between the methods is good over the concentration range normally found in beer fermentations. The following variables in methodology do not affect precision: microwave time in excess of 5 min, sample location in microwave oven, use of plastic (vs glass) weighing vessels, and sample size within a fixed dry weight range. Use of a mechanical pipet reduces precision.

Key words: Analytical method, Dry weight, Microwave drying, Moisture content, Yeast

In our fermentation research, we needed to measure yeast dry weight concentration during fermentation with a method that was fast, precise, and economical of sample. We also required that the method be applicable over the concentration range encountered

during beer fermentation without sample size adjustment and that it be quantitatively related to conventional methods of yeast dry weight measurement. About two years ago we developed a microwave method which, over several hundred determinations, met all of the above requisites.

The advantages of drying small samples of biological materials using microwaves rather than hot air are persuasive; the samples dry faster and with less degradation of the residual solids (2,4,5,7,8,9). These advantages derive directly from the fact that water, far more than other components of biological material, absorbs microwave radiation and converts it to heat. Fat absorbs about a hundredth of the microwave radiation that water does, and other cell components such as protein and carbohydrate absorb far less. Fresh steak, for example, absorbs 10^4 times more microwave energy than does lyophilized steak (3).

In relatively thin samples, water is heated internally by microwaves as readily as at the surface; therefore, evaporation is not limited, as with hot air drying, by the inward diffusion of heat. The higher surface temperature associated with hot air drying causes "skinning," which further impedes evaporation. Furthermore, the extent of microwave heating is directly proportional to the amount of water present (6); therefore excessive heating, with increased degradation of the solids, is avoided. In fact, the lethality

¹ Presented at the 46th Annual Meeting, Minneapolis, MN, May 1980.

² Present address: S. C. Johnson and Company, Racine, WI.

of microwave radiation to microorganisms is directly proportional to their moisture content; lyophilized yeast, bacteria, and viruses are unaffected (10).

EXPERIMENTAL

Apparatus

A microwave oven capable of about 600–800 W of power output at 2,450 MHz was used. It was equipped with a stirrer and a no-load protector.

A stirrer is a device to distribute microwave energy uniformly in the cavity. A no-load protector minimizes damage to the magnetron tube when the oven is operated without "high-loss" (watery) material in it. Almost all commercial units designed for home use meet these criteria. We have successfully used a Litton 520 and a General Electric JET-88 oven in our laboratory.

Amicon filter membranes, 25 mm in diameter with a 0.45- μ m pore size, were used in Millipore "Swinnex" filter holders. Plastic syringes of 12-ml capacity ("Monoject," from Scientific Products) were used for convenience and safety.

Weighing dishes (Weigh boat, No. B2045-5, of polystyrene, from Scientific Products) were approximately 4.5 \times 4.5 cm square. Our experience showed that covered Pyrex weighing bottles could be substituted for the plastic weighing dishes, with both the advantages and disadvantages of a closed glass container. Metal containers could not be used because they reflect microwave radiation.

The analytical balance was capable of weighing to the nearest 0.01 mg.

Procedure

A plastic weighing dish, containing a filter membrane and the gasket from a filter holder, was tared. The membrane and gasket

were assembled into the filter holder, which was then attached to a syringe. Ten milliliters of degassed fermenting beer were measured into the syringe with a volumetric pipet and forced through the filter with the syringe plunger. The entrapped yeast was washed by forcing 10 ml of deionized water through the filter assembly. Because the filter membrane was supported only on its exit surface, drawing a vacuum by pulling up the syringe plunger would rupture the membrane. Therefore, the syringe was separated from the filter assembly before the plunger was extracted for procedures such as adding rinse water.

The filter unit was then carefully disassembled, and the gasket, membrane, and intact yeast were transferred with forceps to the tared weighing dish. The dish was transferred to the microwave oven and dried for 5 min at a setting that delivered a steady 600–800 W of microwave energy. The weighing dish was transferred to a desiccator for about 15 min before being weighed. The dry weight concentration in grams per liter (or milligrams per milliliter) was the weight difference divided by the sample volume.

When large numbers of samples were handled simultaneously, tared weighing dishes were matched with filter units so that tared membranes and gaskets could be rematched with their weighing dishes after filtration.

Comparison with Hot-Air Oven Drying Method

The rapid method was compared to a typical hot-air oven method (1), using the following procedure. Aliquots (10–30 ml) of fermenting beer were centrifuged in 50-ml tubes at 5,000 rpm (about 2,500 \times g) for 5 min. The supernatant was discarded, and the sedimented yeast resuspended in deionized water, then recentrifuged. The yeast pellets were quantitatively washed into glass

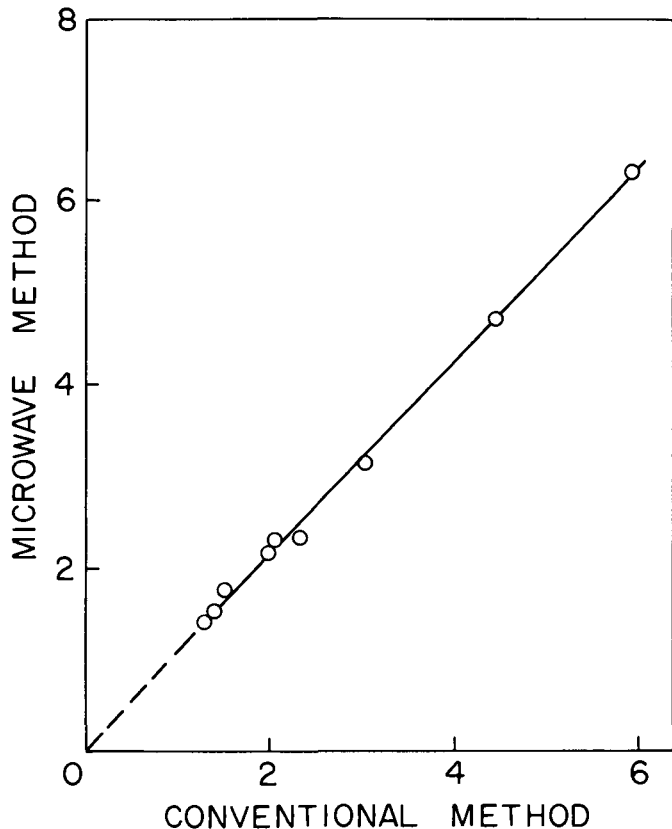


Fig. 1. Regression line for yeast dry weight concentration (grams per liter) in fermenting beer, determined by both microwave method and conventional method. Sample points ($n = 9$) are means of 2–3 replicate determinations by each method.

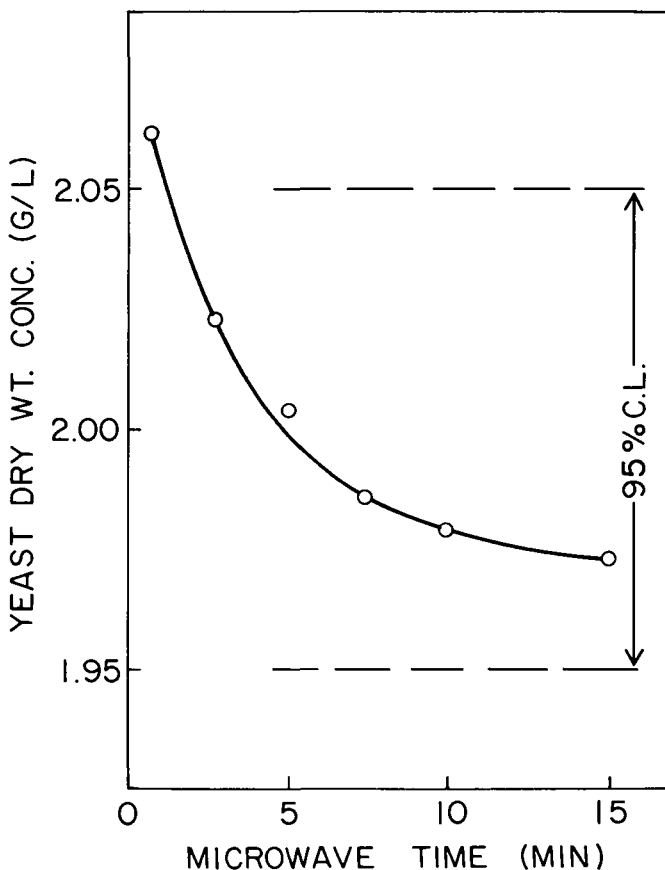


Fig. 2. Weight loss, as yeast dry weight concentration (grams per liter) at various times of microwave drying. Data points are means of duplicates. 95% confidence limits (C. L.) = mean of 5-min dry weights \pm (1.96 \times standard deviation of 5-min mean) = 2.003 \pm 0.055 g/L.

weighing bottles, which had been previously tared after at least 2 hr at 105°C. The samples were dried overnight (about 18 hr) in a 105 ± 2°C oven before being cooled in a desiccator and being covered and weighed.

Dry weight concentrations were determined simultaneously by the two methods, using aliquots of the same sample. This simultaneous comparison was repeated on samples at various fermentation times.

RESULTS AND DISCUSSION

Analysis of the data showed no difference in the precision of the two methods. The coefficient of variation (c.v.) was 1.0% for the conventional method and 1.3% for the microwave method. Regression analysis showed a significant difference in the values obtained by the two methods, with the microwave values being greater. The regression line, shown in Fig. 1, has the equation:

$$Y = 1.051X + 0.046$$

in which:

Y = yeast (grams per liter) by the microwave method

X = yeast (grams per liter) by the conventional method (1).

A *t*-test of the slope (coefficient of X) showed a significant difference from unity ($P = 0.05$), which indicates that the difference in weight by the two methods is real. The high correlation coefficient of the regression line ($r = 0.999$) indicates good correlation of the two methods at all yeast concentrations.

Leonhardt et al (7) found that the percent solids of microwave-

dried pressed yeast was greater than that of hot-air dried yeast (12) by about the same amount as described here. They attributed the greater weight to the fact that less heat is developed as the water is evaporated. Because even prolonged microwave irradiation of the samples (30 min) did not reduce the dry weight concentration to that value obtained in the hot air oven, we think that the greater weight is due to less degradation of yeast solids.

Effect of Experimental Variables

Effect of Microwave Oven Time. All visible water evaporated within seconds after the samples were placed in the microwave oven. The time required to completely dry them was determined by weighing replicate samples periodically during an extended microwave drying period. The results of one of these experiments is shown in Fig. 2. The weight (expressed as dry weight concentration) was assumed to be constant at 15 min (ie, 0% moisture). The 95% confidence limits of the weight at 5 min shown in Fig. 2, indicate that the experimental error is greater than the error introduced by incomplete drying.

The percent moistures at the other weighing times were calculated and plotted on semilog paper (Fig. 3). The samples contained only about 5% moisture after 45 sec in the oven, confirming that the most of the water evaporates quickly. The rate of decrease of the residual moisture is clearly exponential. Two other experiments gave results similar to those shown in Figs. 2 and 3, even at very different dry weight concentrations.

Effect of Sample Location in the Oven. The microwave oven is actually a resonant cavity, and uneven wave distributions, or modes, can occur. Although nearly all modern ovens have mode stirrers, some oven locations may receive less microwave energy than others. To determine if this could cause uneven drying, 21 replicate samples were distributed uniformly throughout the oven and simultaneously irradiated for 5 min. The precision of the resulting dry weight measurements, expressed as the c.v., was 2.0%. This demonstrates good uniformity, regardless of sample position in the oven. We recommend that this type of test be done with any microwave oven considered for sample drying.

Copson (3) suggests that raising the samples about 1 cm from the oven floor with nonmetallic supports facilitates drying and avoids heat from the metal surface.

Effect of Apparatus Substitutions. The equipment was constructed with all low-loss materials, that is, materials relatively unaffected by the microwave radiation. To ensure that this was the case, several assemblies of plastic weighing dish, gasket, and membrane filter were exposed for 15 min in the oven; no change in weight was found. Most plastics and elastomers are low-loss materials, as shown in Table I, and should be satisfactory.

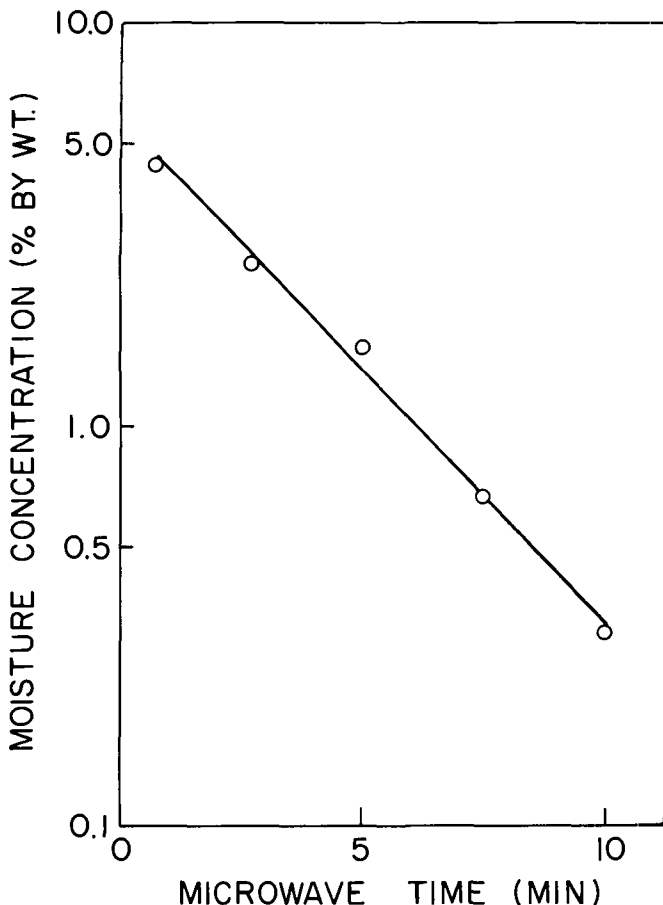


Fig. 3. Semilog plot of moisture content, as percent water, at various times of microwave drying. If the sample is assumed to be dry (0% H₂O) after 15 min, the correlation coefficient of the regression line is 0.997.

TABLE I
Relative Microwave Absorption of Various Materials^a

Material	Percent Relative Absorption at 25°C ^b
Plastic	
Polyvinyl chloride	0.13
Polystyrene	0.006
Polyethylene	0.006
Teflon®	0.003
Plexiglass®	0.13
Cellulose acetate	0.73
Rubber	
Natural rubber	0.054
Butyl rubber	0.02
Silicone rubber	0.48
Neoprene®	1.14
Glasses and Ceramics	
Pyrex®	0.20
Fused quartz	0.002
Porcelain (Coors)	0.069

^a Calculated from tables in Von Hippel (11).

^b Relative to water at 25°C.

We preferred using disposable plastic weighing dishes rather than covered glass weighing bottles, although we recognized that under certain circumstances the latter could prove superior. We compared the two types of containers (under conditions of low relative humidity) and found that the precision was about 1% c.v. or less when using volumetric pipets (Table II). When we used a mechanical pipet (Oxford "Macro Set"), the precision was about 4% whether glass or plastic containers were employed. A c.v. of less than 5% may justify the use of the faster mechanical pipets.

Effect of Sample Size. The precision of the microwave method (c.v. = 1.3%) was determined for sample dry weights of 10–70 mg, which required 10-ml samples of a normal beer fermentation. The precision was the same for all samples within this dry weight range, regardless of sample volume. Samples above this weight range could be difficult to remove quantitatively from the filter holder.

Scope of the Method

The method is characterized by the isolation of yeast on membrane filters and the removal of water by microwave drying. It can be extensively modified within this framework; for example, vacuum membrane filtration should be as satisfactory as pressure filtration. Shorter microwave times (1–2 min) or mechanical pipets can be substituted, although precision may be adversely affected.

The microwave oven could probably be substituted with equal advantage in many of the drying methods presently using a hot-air oven, such as for grains, hops, spent grains, and yeast slurry.

ACKNOWLEDGMENTS

We wish to thank James Munroe and Duane Malek for their help with the statistical calculations. We also thank the officers of the Miller Brewing Company for permission to publish this work.

TABLE II
Effect of Apparatus Modification upon Microwave Method Precision^a

Pipet Type	Weighing Vessel	
	Plastic	Glass
Volumetric	1.2 (5) ^b	0.4 (3)
Mechanical ^c	3.5 (5)	4.5 (5)

^a Expressed as coefficient of variation in percent.

^b Figures in parentheses indicate degrees of freedom.

^c Oxford "Macro Pet."

LITERATURE CITED

1. Association of Official Analytical Chemists. Official Methods of Analysis (11th ed.). Chap. 10.173: Air Oven Method (25). The Association: Washington, DC, 1970.
2. Chevalier, M. *Ind. Aliment. Agric.* 87:243, 1970.
3. Copson, D. A. *Microwave Heating* (2nd ed.). Avi Publishing Co., Inc.: Westport, CT, 1975.
4. Cotterill, O. J., and Delaney, I. *Food Technol.* 13:476, 1959.
5. Gomez, A. M. F., Leonhardt, G. F., Torloni, M., and Borazani, W. J. *Microwave Power* 10(3):266, 1975.
6. Hopkins, A. L. *Ann. N. Y. Acad. Sci.* 85:714, 1960.
7. Leonhardt, G. F., Gomez, A. M. F., Borzani, W., and Torloni, M. J. *Microwave Power* 13(3):235, 1978.
8. Reveron, A. E., Gelman, A. C., and Topps, J. H. *Lab. Prac.* 20(12):943, 1971.
9. Stephenson, M. G., and Gaines, T. P. *Tob. Sci.* 19:51, 1975.
10. Vela, G. R., and Wu, J. F. *Appl. Environ. Microbiol.* 37(3):550, 1979.
11. Von Hippel, A. R. (ed.). *Dielectric Materials and Applications*. Massachusetts Institute of Technology Press: Cambridge, MA, 1954. p. 301.
12. White, J. *Yeast Technology*. Chapman and Hall: London, 1954. p. 144.

[Received May 12, 1980]