# Influence of Dough-Making Conditions on the Concentration of Individual Sugars and Their Utilization During Fermentation[1]

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**ABSTRACT**

Experiments were performed using high-performance liquid chromatography for analysis of sugars during dough mixing. A Chopin rheofermentometer was used to analyze gas production and dough height during dough fermentation. The maltose content of dough increased 47- and 59-fold, respectively, after 5 and 15 min of mixing. However, these results varied from one flour to another according to their level of damaged starch. The maltose content was dependent upon the mixing time and speed and upon the level of exogenous α-amylase, whereas dough hydration and mixing temperature did not affect the final content of maltose. Without yeast, the fructose content of dough increased two- and threefold, respectively, after 5 and 15 min of mixing, owing to the presence of a fructosidase in the flour. With yeast, the fructose content of dough increased 6- and 10-fold after the same mixing times, whereas the sucrose decreased to zero after 5 min of mixing. These last results are obviously related to the high invertase activity of yeast. During fermentation, the glucose content largely decreased compared to that of fructose because the former hexose is a preferred substrate for yeast. The available sugars do not seem to be a limiting factor of fermentation because further increases in maltose content between 10 and 15 min of mixing (or the addition of exogenous α-amylase) did not result in an increase of gas production during fermentation.

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Historically, the ability of baker's yeast to ferment dough has been related to the amount of fermentable sugars in the flour, including maltose produced from starch hydrolysis (Colin and Belval 1935, Guillemet 1936, Geoffroy 1939). More recently, Lee et al (1959) and Lee and Geddes (1959) followed the variations of sugar concentrations during fermentation. To our knowledge, no report has stressed the importance of mixing conditions on the sugar content of dough. By producing an homogeneous mixture of the constituents, mixing promotes molecular interactions and increases the enzyme-substrate contacts and the product diffusion.

The purpose of this study was to investigate how the dough components (flour, water, yeast, and α-amylase) and mixing conditions (time, speed, and temperature) can affect the mono- and disaccharide concentration and gas production.

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## MATERIALS AND METHODS

### Materials

Three flours were used in these experiments: a patent white bread flour obtained from La Française de Meunerie (Corbeil, France); and two other flours milled from 25 kg of pure wheat cultivars (Soissons and Récital) using a homemade semi-industrial mill (Ecole Nationale Supérieure de Meunerie et des Industries Céréalières, Paris, France). Some analyses of these flours are given in Table I.

The baker's instant dry yeast was from Société Lesaffre. Fungal α-amylase EC 3.2.1.1 (P500) and glucose oxidase EC 1.1.3.4 (100,000 U/g) were from Gist-Brocades and Sigma, respectively. Fructose, glucose, sucrose, maltose (Merck), and raffinose (Sigma) were used without further purification.

### Mixing Conditions

Doughs were prepared by adding 63, 78, and 93 ml of salted water to 150 g of flour. The hydration levels were 39.4, 43.4, and 46.9% (w/w), respectively. The final concentration of NaCl was kept constant at 3.85% (w/w) of dry dough. Two other experiments were performed at the medium hydration level

(43.4%) with 1 g of baker's yeast or 50 mg of α-amylase.

The mixing was performed at two temperatures (20 and 30°C) and two mixing speeds (50 and 100 rpm) using the micromixer of the Chopin alveograph.

The dough was prepared as follows: 15 min of mixing, 30 min of resting, 5 min of mixing, and lastly, 30 min of resting. During the first mixing period, an aliquot was taken every 5 min. An aliquot was taken at the beginning and end of the second mixing period. Analyses were also performed on the initial flour and on the dough at the end of the experiment.

### Mono- and Disaccharide Extraction

Aliquots of flour (5 g) or dough (2 g) were suspended in 100 ml of boiling 80% (v/v) aqueous ethanol and maintained at 80°C for 30 min to inactivate enzymes and extract sugars (Mercier and Tollier 1984). After centrifugation (15,000 × *g*, 30 min), the supernatant was evaporated to dryness under partial vacuum at 40°C. The dry extract was dissolved in water (25 ml) and filtered on a Whatman membrane filter (0.45 μm). A filtrate portion (1 ml) was evaporated, and the dry residue was dissolved in a mixture of acetonitrile and water (60:40, v/v) for the quantitative separation and determination of sugars by high-performance liquid chromatography (HPLC).

### Mono- and Disaccharide Determination

Carbohydrate analysis was performed using a liquid chromatograph equipped with a differential refractometer (Erma 7511, sensitivity $5 \times 10^{-6}$ RIU $FS^{-1}$) and integrator (SP4270 Spectraphysics).

A spherisorb $NH_2$ column (4.6 × 250 mm; particle size 5 μm) was used. An isocratic elution was performed at 20°C using a flow rate of 1.2 ml/min. The eluant was a mixture of acetonitrile and water. The composition was 68:32 (v/v) for the glucose and fructose determination, and 75:25 (v/v) for the disaccharides. The sample was injected with a sample loop of 20 μl. The concentrations of carbohydrate standards were between 0.5 and 1.5 mg/ml.

### Acid Hydrolysis of Flour and Total Fructose Determination

Samples of flour (2 g) were boiled under reflux for 3 hr in 100 ml of 0.1*N* $H_2SO_4$. The hydrolysate was cooled and neutralized with 0.1*N* NaOH (Southgate 1969). A 10-ml portion was treated with 50 mg of glucose oxidase for 12 hr at 37°C and then filtered on a Whatman membrane filter (0.45 μm) and dried. The residue was dissolved in a mixture of acetonitrile and water (60:40, v/v) for the quantitative determination of fructose by HPLC.

### Measurement of Gas Production and Dough Development

Flour (150 g) was mixed for a fixed time with instant dry yeast (1 g) and a variable amount of salted water. All the dough was placed in the Chopin rheofermentometer chamber at 29.5°C. Total volume of gas production (ml/g) and dough height (mm) under stress of 1.25 kg were automatically determined after 3 hr.

### Expression of Results

All the results were expressed on a dry weight flour basis and represent the mean value of experiments performed in duplicate or triplicate. Data were analyzed using the Student's *t* test for statistical analysis.

### Measurement of Damaged Starch and Water Activity

The level of damaged starch of the flours was automatically determined with the Chopin SD4. Water activity of the dough

**TABLE I**
**Protein, Ash, and Damaged Contents of Flours[a]**

| Flour | Protein | Ash | Damaged Starch |
|---|---|---|---|
| Patent wheat | 11.2 | 0.60 | 13 |
| Soissons | 11.7 | 0.56 | 5.5 |
| Récital | 10.3 | 0.55 | 8.2 |

[a]% on dry weight flour basis.

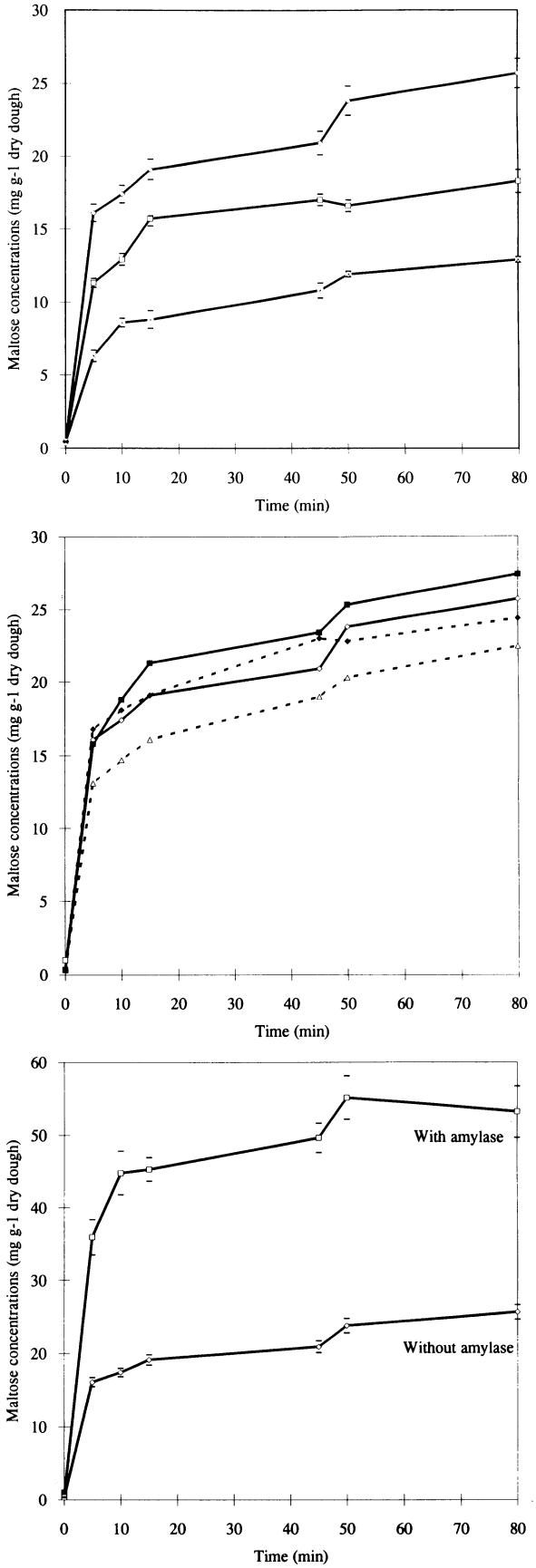


**Fig. 1.** Evolution of maltose concentrations during dough making. Mixing conditions: 43.4% water content, 30°C. **A,** Influence of wheat cultivars. Mixing speed: 50 rpm, without yeast. × = Soissons, □ = Récital, ◇ = patent flour. **B,** Influence of mixing speed. Open symbols = 50 rpm, filled symbols = 100 rpm; dashed lines = with yeast, solid lines = without yeast. **C,** Influence of exogenous α-amylase (20U SKB/g of dry flour) on patent flour without yeast. Mixing speed: 50 rpm.

was evaluated with a dew point electronic humidity meter (Aqualab CX-II).

## RESULTS AND DISCUSSION

### Quantitative Analysis of Sugars in the Flours

The differences in sugar composition between the patent flour and the flours obtained from the two cultivars of wheat were not significant (data not shown).

The concentrations of soluble mono- and disaccharides in dry flours are (in decreasing order): sucrose (2.18 ± 0.17 mg/g), raffinose (0.84 ± 0.08 mg/g), maltose (0.40 ± 0.03 mg/g), fructose (0.34 ± 0.02 mg/g), and glucose (0.29 ± 0.03 mg/g). These values are in agreement with those reported by others (Colin and Belval 1935, Geoffroy 1939, Koch et al 1951, Cerning and Guilbot 1974, Lineback and Rasper 1988).

Thus, the amount of soluble and fermentable carbohydrates in flour is higher than 4 mg/g. Sucrose is most abundant, accounting for more than 50% of the total soluble sugars, which is in agreement of the results of Uglade and Jenner (1990).

After hydrolysis, the mean total fructose content (free and initially present in sucrose and other oligosaccharides) was higher than 7 mg/g (7.2 ± 0.26 mg/g).

### Effect of Yeast, Mixing Time, and Mixing Speed on Dough Sugar Content

The maltose production began as soon as water was added to the flour, and it was very rapid during the first minutes of mixing (Fig. 1A). The amylolysis of starch was much more important during the first mixing period than it was during the resting period. Thus, the maltose content of the patent wheat flour increased 47- and 59-fold after 5 and 15 min of mixing, respectively, whereas it increased <10% during the subsequent resting period. When the mixing speed was doubled, the maltose content of dough increased by 10–15% (Fig. 1B).

A similar maltose production pattern was found for the flours obtained from Soissons and Récital cultivars (Fig. 1A). However, in similar conditions, the amount of maltose in doughs was always in the increasing order: Soissons, Récital, and patent wheat. This result cannot be explained by differences in the endogenous α-amylolytic activities among the flours, because they exhibited the same Hagberg falling number (400 sec). The differences in maltose production are obviously related to the initial level of damaged starch of the three flours (Table I). The values fluctuated slightly (~3 mg/g), so sucrose was not affected by mixing and resting conditions. A small amount of glucose was produced by flour amylases with a maximal value close to 2 mg/g. Similarly for the fructose, the twofold increase after 5 min of mixing and the threefold increase after 15 min of mixing were probably related to a fructosidase present in the flour (Table II).

### Yeasted Dough

The evolution of maltose in yeasted doughs was similar to that found with unyeasted doughs. Doubling the mixing speed resulted in a 15% increase of the maltose content of yeasted dough (Fig. 1B). Differences were apparent for the other sugars. The high invertase activity of yeast is obviously responsible for the rapid disappearance of sucrose as well as the greater increase in fructose production for the yeasted doughs (Table II).

The glucose content was higher during the first mixing period but became lower during the subsequent resting and mixing periods (Table II). This can be easily explained, as glucose is the preferred substrate of baker's yeast during fermentation.

The amount of fermentable sugars as well as gas production increased with the mixing time (Table III). The dough development under a stress of 1.25 kg was optimal at a mixing time of 10 min. Gas production of dough increased between 5 and 10 min of mixing. It remained almost constant after 15 min of mixing, although the maltose content of dough increased (Fig. 1B, Table III). Therefore, it appears that the amount of available maltose was not the limiting factor of fermentation; more likely it was the metabolic capacity of yeast.

### Effect of Dough Hydration

In the dough-water concentration range examined, no significant changes in concentrations of maltose, glucose, and fructose were observed. For the conditions mentioned here, the thermodynamic activity of water remained almost constant and close to 1; the values obtained with the Aqualab apparatus after 10 min of mixing were 0.987, 0.990, and 0.996, respectively, for 39.4,

**TABLE II**
**Sucrose, Fructose, and Glucose Concentrations (mg/g of dry dough) During Dough-Making of Patent Wheat Flour[a,b]**

| Procedure | Time Elapsed (min) | Sucrose | | Fructose | | Glucose | | |
|---|---|---|---|---|---|---|---|---|
| | | Without Yeast | With Yeast[c] | Without Yeast | With Yeast[c] | Without Yeast | With Yeast[c] | With α-Amylase |
| | 0 | 2.35 ± 0.20 | 2.35 ± 0.20 | 0.40 ± 0.05 | 0.40 ± 0.05 | 0.26 ± 0.09 | 0.26 ± 0.09 | 0.26 ± 0.09 |
| Mixing | 5 | 2.98 ± 0.25 a | 0 a | 1.12 ± 0.59 a | 3.77 ± 0.50 a | 0.30 ± 0.30 ab | 1.34 ± 0.29 ab | 2.44 ± 0.71 b |
| Mixing | 10 | 2.84 ± 0.15 a | 0 a | 1.45 ± 0.50 a | 4.66 ± 0.54 a | 0.84 ± 0.14 a | 1.45 ± 0.74 b | 4.17 ± 0.37 ab |
| Mixing | 15 | 2.66 ± 0.16 a | 0 a | 1.39 ± 0.50 a | 5.54 ± 0.82 a | 1.70 ± 0.6 a | 1.56 ± 0.24 b | 3.83 ± 0.41 ab |
| Resting | 45 | 2.93 ± 0.20 a | 0 a | 2.35 ± 0.24 a | 5.81 ± 0.58 a | 1.89 ± 0.38 a | 1.72 ± 0.72 b | 5.89 ± 0.48 ab |
| Mixing | 50 | 3.22 ± 0.15 a | 0 a | 2.18 ± 0.15 a | 5.89 ± 0.69 a | 1.90 ± 0.29 a | 1.32 ± 0.84 b | 5.85 ± 0.76 ab |
| Resting | 80 | 2.65 ± 0.48 a | 0 a | 1.09 ± 0.63 a | 4.36 ± 0.58 a | 1.59 ± 0.72 a | 1.21 ± 0.42 b | 6.71 ± 0.34 ab |

[a] Mixing conditions: water content of 43.4%, 30°C, 50 rpm.
[b] Means ± standard deviation with the same letters differ horizontally at $P < 0.05$.
[c] With 1 g of baker's yeast.

**TABLE III**
**Gas Production and Dough Height Measured by a Rheofermentometer at Two Temperatures and Three Water Contents, With and Without Exogeneous α-Amylase[a]**

| Mixing Conditions | Gas Production (ml/g of dry dough) | | | Dough Height (μm/g of dry dough) | | |
|---|---|---|---|---|---|---|
| | 5 min | 10 min | 15 min | 5 min | 10 min | 15 min |
| 39.4% water, 30°C, 50 rpm | 6.58 ± 0.33 a | 7.12 ± 0.13 a | 7.21 ± 0.07 ac | 83.7 ± 2.4 ab | 82.9 ± 5.5 a | 92.0 ± 5.5 a–c |
| 43.4% water, 30°C, 50 rpm | 7.40 ± 0.01 a | 7.79 ± 0.04 ab | 8.01 ± 0.02 ab | 145 ± 12 a | 152 ± 7 a | 140 ± 7 a |
| 100 rpm | 7.95 ± 0.05 a | 7.94 | 7.73 ± 0.10 a | 172 ± 7 b | 144 | 138 ± 9 b |
| 50 rpm + amylase | 7.01 ± 0.04 a | 7.23 ± 0.04 b | 7.52 ± 0.11 ab | 194 ± 3 ac | 219 ± 29 a | 200 ± 2 ab |
| 43.4% water, 20°C, 50 rpm | 5.65 ± 0.10 a | 5.88 ± 0.04 ab | 6.05 ± 0.06 ab | 156 ± 1 c | 148 ± 1 a | 149 ± 1 c |
| 46.9% water, 30°C, 50 rpm | 7.71 ± 0.00 a | 8.04 ± 0.01 ab | 8.09 ± 0.10 c | 249 ± 11 a–c | 250 ± 3 a | 254 ± 4 a–c |

[a] Means ± standard deviation with the same letters differ vertically at $P < 0.05$.

TABLE IV
Effect of the Dough Hydration on the Relative Increases in Gas Production and Dough Height at Three Mixing Times

| | 39.4% Water | | 43.4% Water | | 46.9% Water | |
|---|---|---|---|---|---|---|
| Mixing time, min | Gas Production, % | Dough Height, % | Gas Production, % | Dough Height, % | Gas Production, % | Dough Height, % |
| 5 | 100.0 | 100.0 | 112.5 | 173.6 | 117.1 | 290.7 |
| 10 | 108.2 | 98.8 | 118.4 | 181.2 | 122.2 | 298.1 |
| 15 | 109.57 | 109.9 | 121.7 | 167.1 | 122.95 | 302.7 |

43.4, and 46.9% water content. Therefore, in this limited range of water activity, changes in hydration did not seem to affect the enzymatic activities involved in the maltose, sucrose, glucose, and fructose evolutions.

However, gas production and dough development under stress were significantly affected by the hydration level of dough (Table IV). Gas production was optimum after 10 min of mixing, and it increased with the hydration level. The highest variations were between 39.4 and 43.4% water content. Therefore, using these substrates, no direct relationship was found between the amount of fermentable sugars in dough and the yeast capacity. The dough development under stress was also dependent upon the dough-water content, but it was not affected by the mixing time. When water hydration increased from 39.4 to 43.4%, dough development doubled. A similar increase was observed when water hydration increased from 43.4 to 46.9% (Table IV).

### Effect of Mixing and Resting Temperature

The amount of carbohydrates was not affected at mixing and resting temperatures of 20 and 30°C. However, gas production decreased by ~20% with temperature (Table III), even though the difference in temperature was effective only during the first mixing period. In all cases, the 3-hr fermentation was performed at 29.5°C in the rheofermentometer chamber.

### Effect of Fungal α-Amylase

The introduction of 20U SKB/g of dry flour doubled the maltose content and increased the glucose content of dough fourfold (Fig. 1C, Table III). The levels of sucrose and fructose were not affected (results not shown). Although the amount of available substrate increased, gas production was significantly lower (Table III). Such a result could be due to an osmotic effect, which is susceptible to decreases in the metabolic capacities of yeast cells. Lastly, dough development under stress increased almost 40% for dough supplemented with exogenous α-amylase (Table III).

## CONCLUSIONS

The carbohydrate production rate appeared very rapid in the first minutes of mixing and became much slower during the subsequent resting period. After the initial increase of water content, the mechanical work is obviously of great importance for the frequency of the enzyme-substrate contacts. Maltose production was dependent upon wheat cultivar and the level of damaged starch and amylolytic activity.

The sugar amount increased with mixing time and speed. It also increased in the presence of exogenous α-amylase. Conversely, dough hydration and temperature during mixing had no significant effect. No direct relationship was found between the fermentation power of yeast (measured during a 3-hr period) and the amount of available sugars.

The maximal dough development under stress was influenced by water concentration and α-amylase but not by time and temperature of mixing. It appears that water plays a prominent role in dough structure.

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