EFFECT OF CERTAIN NUTRIENTS ON THE GAS PRODUCED IN PREFERMENTS[1]

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ABSTRACT

A synthetic mixture of nutrients plus a buffer gives a yeast fermentation rate equal to that obtained with a flour. A mixture containing ammonium ion, phosphate ion, magnesium ion, sulfate ion, and potassium ion was required for maximum rate. Omitting any of those five ions retarded carbon dioxide production. Other ions, such as iron, zinc, copper, and manganese, and concentrated tap water had no effect on gassing rate. Chelating agents such as ethylenediamine tetraacetic acid likewise had no effect on gassing rate. Flour and/or nonfat dried milk had excellent buffering capacity. Fermentation rate was essentially equal with 60 or 100% flour in the brew. At least 7% sugar was necessary for a system containing no flour to have maximum fermentation activity for 4 hr.

Studies (1–3) have shown that a solution containing potassium, magnesium, phosphate, and sulfate ions, thiamine, pyridoxine, nicotinic acid, a source of yeast-available nitrogen (asparagine), sugar, and a buffer supported a fermentation rate equal to that in a flour suspension fortified with available nitrogen and excess sugar. In their classic paper, Atkin *et al.* (4) showed the effect of each of the above factors on fermentation. Yeast fermentation has an absolute dependence on inorganic phosphate, which is involved in high-energy bond formation and activation of substrates. Magnesium is a cofactor for all kinases (ATP:substrate phosphotransferase). The aldolases of yeasts require K^+ or Na^+ and divalent Mg^{+2} ion for activity. The conversion of pyruvate to acetaldehyde is irreversible and requires thiamine pyrophosphate and Mg^{+2} as indispensable cofactors.

Bakers are using liquid-preferment systems to reduce fermentation times. Our goal was to establish the minimum quantity of each of those nutrients required for maximum fermentation activity, and to assess the significance of the results on baking systems.

MATERIALS AND METHODS

Flour used was an untreated commercial hard winter wheat flour containing 11.9% protein and 0.40% ash. Gassing powers were determined at 30°C with gauge-type pressure meters (National Mfg. Co., Lincoln, Nebr.). The doughs (10 g flour) contained 10 ml water, 0.6 g sugar, 0.15 g salt, 0.2 mg (20 ppm) potassium bromate, and 0.2 g baker's compressed yeast. Standard deviation for gassing power was 30 mm. Chemicals used were reagent grade.

RESULTS AND DISCUSSION

Materials Required for Optimum Activity

Our preliminary investigations confirmed the conclusion of Atkin *et al.* (4) that a synthetic mixture of nutrients would give a yeast fermentation rate equal to

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that obtained with flour. Diammonium phosphate was as good as asparagine as a yeast-available nitrogen source. Contrary to an earlier report (4), vitamins had little, if any, effect on fermentation rate, probably because they were added to the feed during yeast production (5).

The formulation per 10 ml total volume of the modified synthetic mixture was:

$(NH_4)_2HPO_4$	= 0.02 g
$MgSO_4 \cdot 7H_2O$	= 0.014 g
KCl	= 0.006 g
Thiamine·HCl	= 0.00003 g
Pyridoxine·HCl	= 0.00003 g
Citrate buffer (0.264*M*), pH 5.4	= 1 ml

The synthetic mixture plus 0.60 g/10 ml sugar gave a gas production rate equal to flour + nitrogen source for the first 3 hr, then the rate with the synthetic

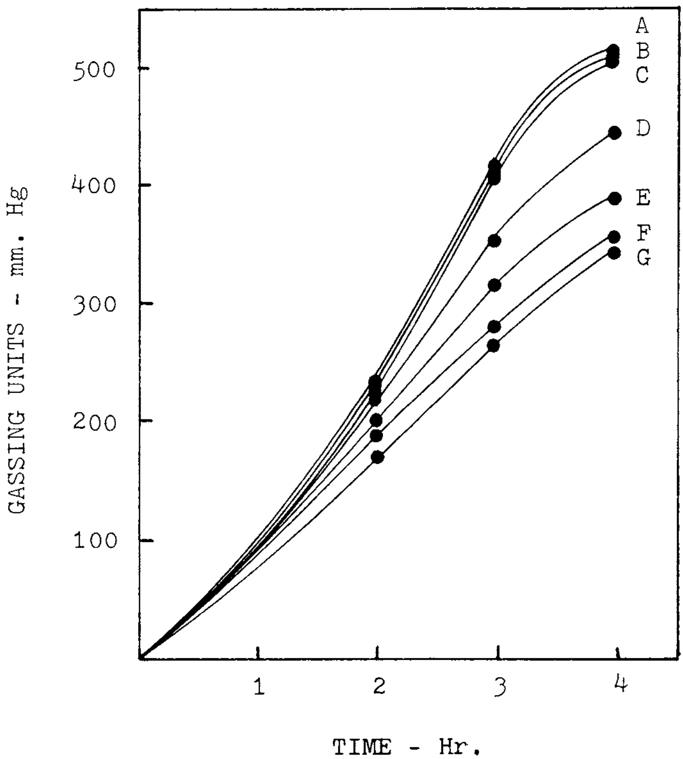

Fig. 1. Gas production with a synthetic mixture containing (per 10 ml): NaH_2PO_4 (6.5 mg); $MgCl_2$ (4.2 mg); Na_2SO_4 (12 mg); KCl (3 mg); thiamine·HCl (0.03 mg); pyridoxine·HCl (0.03 mg); niacin (0.3 mg); 0.0264*M* citrate buffer, pH 5.4; and NH_4Cl at concentrations of A (32 mg), B (24 mg), C (16 mg), D (8 mg), E (4 mg), F (2 mg), and G (0.0 mg).

mixture was lower. Bringing the sugar level to 0.72 g/10 ml maintained a gassing rate equal to that of flour + malt + ammonium ion through 4 hr.

Effect of Ion Concentration on Activity

The effect of concentration of each ion was investigated with the synthetic mixture as a standard. Each ion's concentration was reduced while other ions and the buffer were held constant. Thus, to study the effect of ammonium ion concentration, diammonium phosphate was removed from the mixture and replaced by ammonium chloride and an equivalent amount (based on phosphate ion) of monosodium phosphate. Ammonium chloride concentration was varied (Fig. 1) to show the effect of ammonium ion on gas production. Completely removing ammonium ion gave a gassing power of 340 mm of Hg at 4 hr, compared with 510 mm with the complete mixture. When the ammonium ion was held constant at the level in the control mixture and phosphate ion was varied, similar results were obtained (Fig. 2).

To study the effect of magnesium ion (Mg^{+2}) and sulfate ion ($SO_4^{=}$), we replaced

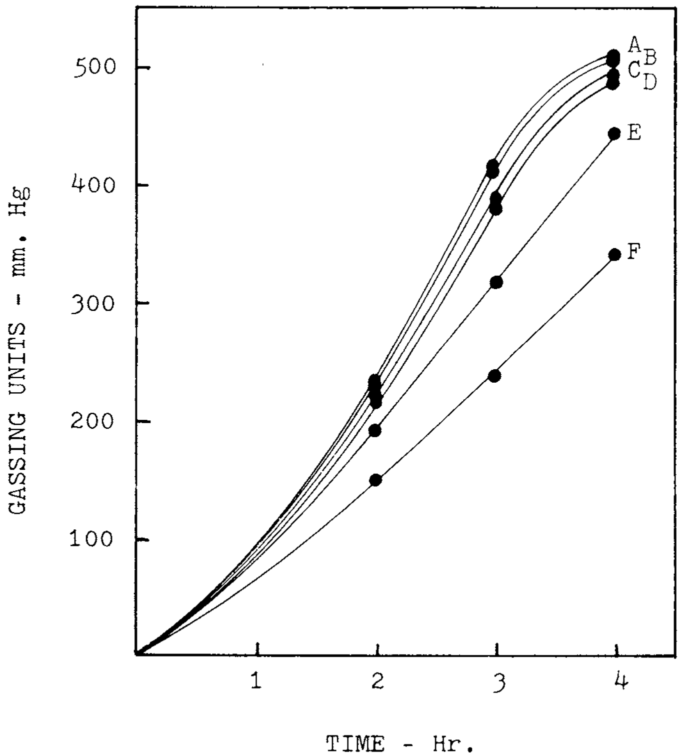

Fig. 2. Gas production for a synthetic mixture containing (per 10 ml): $MgCl_2$ (4.2 mg); Na_2SO_4 (12 mg); KCl (3 mg); NH_4Cl (16 mg); thiamine·HCl (0.03 mg); pyridoxine·HCl (0.03 mg); niacin (0.3 mg); 0.0264*M* citrate buffer, pH 5.4; and NaH_2PO_4 at concentrations of A (13 mg), B (6.5 mg), C (3.2 mg), D (1.6 mg), E (0.8 mg), and F (0.0 mg).

magnesium sulfate ($MgSO_4$) with magnesium chloride ($MgCl_2$) and sodium sulfate (Na_2SO_4). The effect of magnesium chloride on the 4-hr gassing value was particularly large, varying from 255 with no magnesium chloride to 510 at optimum (Fig. 3). The effect of sodium sulfate on gassing power varied from 420 mm at 0% concentration to 510 mm at optimum (Fig. 4). Omitting potassium chloride from the mixture gave 445 mm at 4 hr compared with 510 mm for the complete mixture (Fig. 5). It was clear that omitting any of those five ions retarded carbon dioxide production. Magnesium ion appears to have more effect than any of the others; however, all are indispensable. A mixture containing ammonium chloride, 0.016 g/10 ml; monosodium phosphate, 0.0065 g/10 ml; magnesium chloride, 0.0021 g/10 ml; sodium sulfate, as little as 0.0005 g/10 ml; and potassium chloride, 0.0058 g/10 ml; was required for maximum activity.

Effect of Certain Other Ions and Chelating Agents

The presence of trace minerals (such as Fe, Zn, Cu, Mn) and the vitamin inositol has been reported to stimulate yeast growth (6). No significant increase

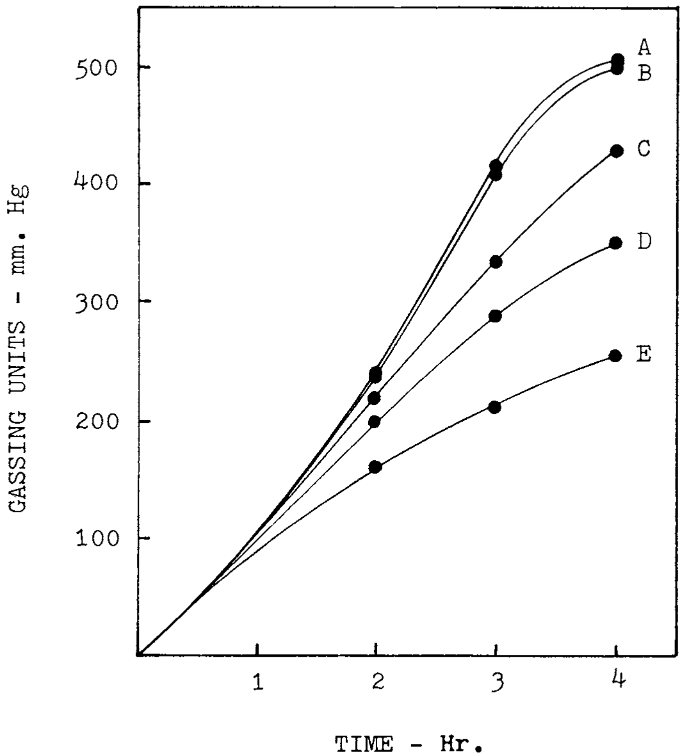

Fig. 3. Gas production for a synthetic mixture containing (per 10 ml): NH_4Cl (16 mg); NaH_2PO_4 (6.5 mg); Na_2SO_4 (12 mg), KCl (3 mg); thiamine·HCl (0.03 mg); pyridoxine·HCl (0.03 mg); niacin (0.3 mg); 0.0264*M* citrate buffer, pH 5.4; and $MgCl_2$ at concentrations of A (4.2 mg), B (2.1 mg), C (1.1 mg), D (0.5 mg), and E (0.0 mg).

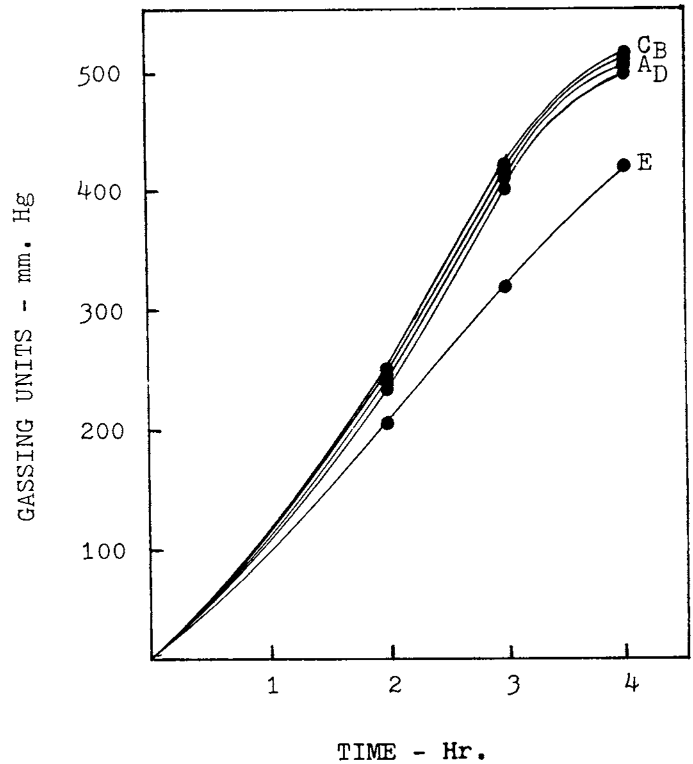

Fig. 4. Gas production for a synthetic mixture containing (per 10 ml): NH_4Cl (16 mg); NaH_2PO_4 (6.5 mg); $MgCl_2$ (4.2 mg); KCl (3 mg); thiamine·HCl (0.03 mg); pyridoxine·HCl (0.03 mg); niacin (0.3 mg); 0.0264*M* citrate buffer, pH 5.4; and Na_2SO_4 at concentrations of A (4.1 mg), B (2.04 mg), C (1.02 mg), D (0.5 mg), and E (0.0 mg).

TABLE I
Effects of Ethylenediamine Tetraacetic Acid, Sodium Phytate, Polyphosphate, Fluoride, Nitrate, Silicate, and Calcium Ions in a Synthetic Mixture

	Gassing Units (mm Hg) at		
Treatments	**2 hr**	**3 hr**	**4 hr**
Synthetic mixture[a]	224	391	498
Mixture + EDTA (500 ppm)	220	381	502
Mixture + NaPhytate (500 ppm)	227	386	500
Mixture + polyphosphate (500 ppm)	227	399	502
Mixture + fluoride (3.1 ppm)	224	388	501
Mixture + nitrate (14 ppm)	232	399	505
Mixture + silicate (49 ppm)	229	380	497
Mixture + calcium (78 ppm)	227	394	501

[a]Containing (per 10 ml): sugar (0.72 g); $(NH_4)_2$ HPO_4 (20 mg); $MgSO_4{\cdot}7H_2O$ (14 mg); KCl (6 mg); thiamine·HCl (0.03 mg); pyridoxine·HCl (0.03 mg); and citrate buffer (0.264*M*).

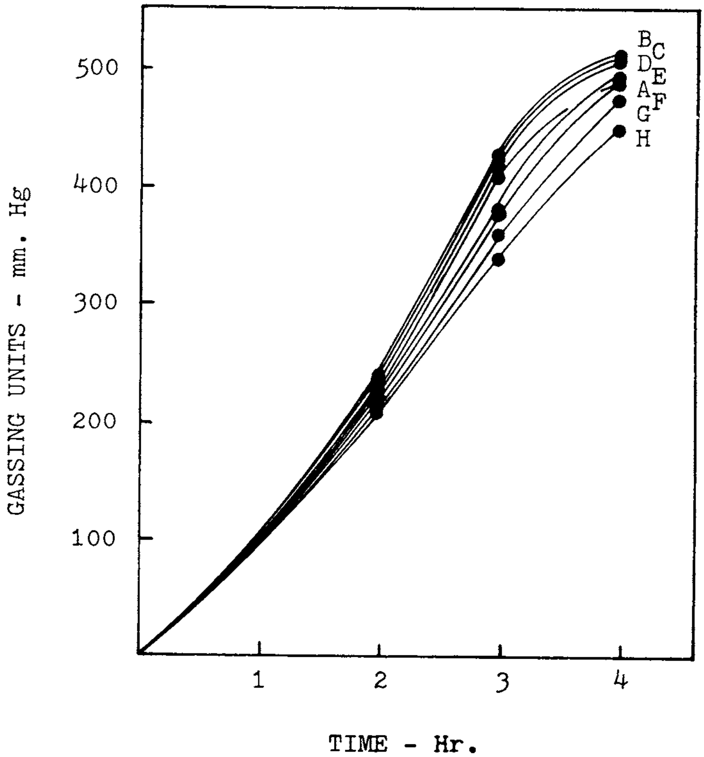

Fig. 5. Gas production for a synthetic mixture containing (per 10 ml): NH_4Cl (16 mg); NaH_2PO_4 (6.5 mg); Na_2SO_4 (12 mg); $MgCl_2$ (4.2 mg); thiamine·HCl (0.03 mg); pyridoxine·HCl (0.03 mg); niacin (0.3 mg); 0.0246*M* citrate buffer, pH 5.4; and KCl at concentrations of A (9.1 mg), B (5.8 mg), C (4.5 mg), D (3.0 mg), E (1.5 mg), F (0.76 mg), G (0.38 mg), and H (0.0 mg).

TABLE II
Effects of Flour Concentrations on Gassing Power and pH after 4-hr Fermentation

% Flour	NFDM		No NFDM	
	G. P.[a]	pH	G. P.	pH
10	386	5.08	340	3.82
20	393	5.11	380	4.18
30	396	5.17	410	4.42
40	422	5.19	418	4.56
50	443	5.23	438	4.68
60	479	5.26	463	4.78
70	477	5.28	463	4.87
100	498	5.14	482	4.88

[a]G. P. = gassing power in mm Hg.

in gas production was found using iron (5 μg), zinc (0.5 μg), copper (0.3 μg), manganese (0.3 μg), and inositol (4 μg), but the fact that the fermentation rate remains constant indicates that trace amounts of those compounds had no harmful effects.

Ethylenediamine tetraacetic acid (EDTA), sodium phytate, and polyphosphates are chelating agents. They were added to the synthetic mixture to determine if a chelating effect would lower gas production. Chelating agents had no deleterious effect on yeast fermentation (Table I).

The effects of tap water and ions in hard water were studied on the synthetic mixture. The maximum reported amount (7) of fluoride (3.1 ppm), nitrate (14 ppm), and silicate (49 ppm) as sodium salts, and calcium (78 ppm) as its chloride gave neither beneficial nor harmful effects on fermentation rate (Table I). When tap water was concentrated 10-fold, 1 ml added to the synthetic mixture had no noticeable effect on fermentation rate.

Significance in Baking Systems

Flour is the primary source of nutrients for yeast fermentation in baking. The effect of flour concentration (10 to 70% of the total flour) was studied, with and

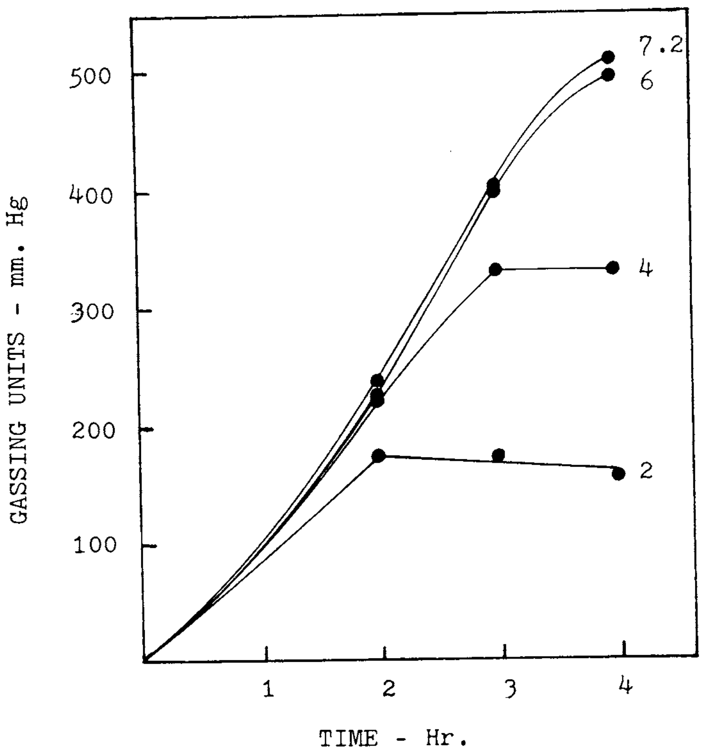

Fig. 6. Effects of sugar concentrations (200, 400, 600, and 720 mg) on gassing power with a synthetic mixture containing (per 10 ml): $(NH_4)_2HPO_4$ (20 mg); $MgSO_4 \cdot 7H_2O$ (14 mg); KCl (6 mg); thiamine·HCl (0.03 mg); pyridoxine·HCl (0.03 mg); niacin (0.3 mg); and 0.0264*M* citrate buffer, pH 5.4.

without NFDM (4%) in the brew (Table II). Sixty per cent flour appeared nearly equal to 100% flour in producing carbon dioxide. The system containing NFDM had a constant pH (approximately 5.10), showing the excellent buffering capacity of milk. Generally, brews contain from 0 to 70% of the flour, with 30% being a common rate of addition. It is clear that lower amounts of flour give slower fermentation rates unless other nutrients are added. Also, low amounts of flour in the brew will give nonoptimal pH, unless the medium is buffered.

Initial fermentation rates were essentially the same for all sugar concentrations studied (from 2 to 7.2% in the synthetic mixture, Fig. 6). Two per cent sugar was exhausted in less than 2 hr, and 4% by 3 hr. When sugar was depleted, no further gas was produced (Fig. 6). Thus, a minimum of 7% sugar was necessary in a system containing no flour for 4 hr of fermentation at maximum activity.

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