

FACTORS AFFECTING ENZYMATIC STARCH HYDROLYSIS IN SUGAR SOLUTIONS

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Abstract

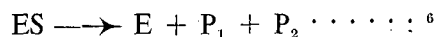
Laboratory investigation of the hydrolysis of starch by bacterial α -amylase in both technical and pure sucrose solutions has been carried out. The influence of reaction time, brix, viscosity, starch concentration, and enzyme dosage is represented in graphical form.

1. Enzyme Reactions

The action of hydrolysing enzyme can in its simplest form be represented by the formation of an enzyme-substrate complex



and the subsequent breakdown of this complex to yield free enzyme and the reaction products



As would be expected, the rates of progress of these reactions are dependent on:

- (a) Concentration of enzyme,
- (b) Concentration of substrate,
- (c) Temperature,
- (d) Viscosity of reacting solution.

A number of other factors also affect enzyme catalysis in general. These include:

- (e) pH,
- (f) Cofactors (coenzymes and activators) which enhance the reaction,
- (g) Enzyme poisons and inhibitors which slow or stop the reaction,
- (h) Thermal inactivation of the enzyme. The magnitude of this effect is in turn reduced by the protective action of certain substances, usually including the substrate. It is known that the enzyme in question (α -amylase derived from *Bacillus subtilis*) is protected in this way by sucrose as well as by starch.
- (i) Substrate composition. The ratio of the two constituent molecular species of starch, amylose and amylopectin, varies according to source. Even starches from the same source have been found to display substantial differences in degree of polymerisation of the linear amylose fraction and in chain length of the branched amylopectin⁷.

2. Scope of Investigation

Some effects of temperature, pH and reaction time on enzymatic starch hydrolysis have been reported³. Subsequent work revealed the importance of the brix

factor and also the interdependence of brix and temperature. A rather more extensive programme was then initiated to assess enzyme performance quantitatively over ranges of brix and temperature likely to obtain in a sugar factory. The results of this investigation are reported here, together with some findings on the effect of independent variation in starch content and in enzyme dosage.

3. Experimental

Test solutions used were mill syrup and a synthetic refined sugar/A.R. soluble starch solution with a ratio of starch to sucrose (0.2%) comparable to that normally found in syrup.

A solution of soluble starch only was used to obtain the curve labelled 'no sucrose' in figure 1, the ratios of starch to solution and enzyme being identical to those for the 7.5 brix level.

Two commercial enzyme preparations were employed.

Reaction was carried out in test tubes of approximately 50 ml capacity, held at constant temperature in a Colara waterbath. Transfer of the tube contents to beakers containing alcohol stopped the reaction by enzyme inactivation. This step also precipitates the remaining starch as the first step of the starch determination method, based on that of Alexander¹.

4. Results

Note that in the work reported in sections 4.1, 4.2 and 4.3, a constant enzyme-to-solids ratio is maintained as would be the case with equivalent-cost application in the process. Variations in brix thus entail proportional changes in concentration of enzyme, starch and any other substances that participate in or affect the reaction.

4.1 Temperature and Brix

Figure 1 shows the effect of temperature at various brix levels for sucrose/starch solutions. The fall-off in activity with increasing temperature, presumably due to thermal inactivation, becomes progressively less severe as the brix rises, and has disappeared (in this temperature range) at 60 brix. Optimum hydrolysis at this point has, however, fallen well below that found at lower brix values.

In Figure 2 the same type of information, this time for syrup, is plotted as starch hydrolysis against brix for three temperature levels. Here the lowest temperature gives significantly inferior results except at very low brix. The 71.1°C curve shows the best optimum performance. This agrees with previously published information⁴.

Curves B and C in Figure 3 represent enzyme performance in sucrose/starch and in syrup solutions respectively, all other conditions being the same. Apart from a lower peak, the curve for syrup shows better performance below about 35 brix. Presumably these differences can be accounted for in terms of the factors mentioned in (d) to (i) in Section 1.

4.2 Viscosity

Values for the viscosity of pure sucrose solutions² were used to derive the plots shown in Figure 4. While the effect on viscosity of the starch present in the solutions used has not been taken into account, it is felt that for comparative purposes this method is permissible. The curves A and B do not intersect as do the corresponding plots against brix A and B in Figure 3. It thus appears that the positive slopes of the 60 and 70 brix curves in Figure 1 can be accounted for in terms of viscosity alone.

4.3 Reaction Time

Figure 5 shows starch hydrolysis in syrup against reaction time, plotted independently for the three temperature levels investigated. At 45 brix, starch hydrolysed after 15 minutes is some 70% of that after 45 minutes, and at 7.5 brix this figure is even higher. It is known that where a thermal inactivation effect is present, reaction velocity falls off progressively to zero. The plot for 7.5 brix at 79.4°C shows complete inactivation to have taken place below 15 minutes reaction time.

4.4 Enzyme Dosage

Increasing weights of enzyme were added to solutions of sucrose/starch and of syrup, each of the same composition throughout. The curves in Figure 6 show quite different profiles for the two test solutions. Performance in syrup is superior at lower levels of dosage, as with brix (Figure 3).

Note that the enzyme dosage axis is logarithmic, and that for example to raise the hydrolysis in syrup from 50% to 60% under these conditions would require nearly double the quantity of enzyme.

4.5 Starch/Sucrose Ratio

A fixed weight of enzyme was added to sucrose/starch solutions of the same brix but containing varying quantities of starch. Figure 7 shows little change in per cent starch hydrolysed with increasing concentration of starch. From this it can be inferred that a roughly constant *percentage* as against weight of starch should be degraded in factory juices of different starch content.

5. Discussion

Only a few of the factors mentioned in Section 1 are subject to any degree of control in a sugar mill. Of these, temperature and especially brix have a pronounced effect on the action of commercial bacterial amylases. It appears that previous anomalous findings on the thermostability of these enzymes can be explained in terms of brix.

Investigation of the effect of pH has not been

included in this work. Bruijn and Jennings quote figures for starch hydrolysis at different levels of pH⁵, but no further references to its effect in sugar solutions have been found. It is conceivable that a relationship analogous to the brix/temperature interdependence may exist for pH.

Acknowledgements

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Discussion

Mr. Bruijn: Referring to Figure 6, the author says that the initial high rate of decomposition of the starch is due to the syrup containing calcium. But do the curves cross over at a dosage of 80 mg per kilo?

The conclusion must be that, in this case, starch in the sucrose solution is not the same as starch in syrup. It appears that in industrial sugar solution part of the starch is not easily attacked by the enzyme.

In Figure 2, if the temperature is raised, the optimum shifts to the higher brix, probably due to a reduction in viscosity. Nevertheless, the optimum at lower concentration is higher than the efficiency at higher concentration, so lower temperatures seem to be more favourable overall.

In Figure 6, at 55 brix and an enzyme concentration about the same as is used in factories, only 40% of the available starch is removed, the same as in Figure 2 at a temperature of 71°C, where 85% is removed.

Because the viscosity is high, the only difference between a laboratory experiment and a factory experiment is the circulation.

Therefore, if you have a high brix solution, does circulation make a difference?

Mr. Smith: I did one test in the laboratory to test the effect of agitation. I used both a high speed stirrer with a small blade and a vibro mixer and I found no difference between these and a solution that was stirred vigorously for a few seconds and left standing. So at the brix used, which was 45, there appeared to be very little difference with stirring.

As regards stirring in the reaction vessel in a factory, it may increase the rate of reaction but is it not better to design the vessel so that the juice passes through on the first in first out principle?

As regards the theory that some portion of the starch may not be susceptible to attack, I do not feel that this is shown here. In Figure 6, the curve tails off to a level approaching about 70%, but this does not mean that 30% is not susceptible to reaction. In Figure 2, under different circumstances, we get to 80% at one point and under more favourable conditions we might get up to 100%, as we have done in laboratory work.

Dr. Matic: Figure 5 shows the percentage rather than the amount of starch that is reduced under various conditions.

Why is it that the *percentage*, as against the *weight* of starch produced, appears to stay the same?

Mr. Smith: I cannot give an explanation, although of course the concentration of the starch increases and this will tend to speed up the reaction.

Mr. Cox: Regarding Dr. Matic's query, the reaction exhibits first order kinetics; this means that the reaction has a discrete and definite half-life.

Therefore, any given quantity of reactant will be degraded by a constant percentage in unit time. When

the active sites of the enzyme have become saturated, as would occur with an excess of reactant, the reaction takes on the characteristics of a zero order reaction. In this case the quantity of material degraded in unit time will be constant at any given temperature. The reaction velocity remains constant regardless of the amount of reactant added.

Under the conditions described, however, the reaction clearly exhibits first order characteristics, and on this basis the degradation of a constant percentage of material in unit time under isothermal conditions is in accordance with theory.

Mr. Jennings: The enzyme usage is expressed as mg/kg brix. As we seem doubtful about the brix, I suggest we might express it as mg/kg final production of sugar.

Mr. Smith: Yes, because it could then be related to sugar lost.

For short periods, such as a day or less, it is difficult to relate juice or syrup throughput to sugar tonnage; however, for normal weekly reporting of enzyme consumption, a sugar basis is certainly preferable.

TABLE I
Analytical Data

Mill	Month	Dry solids %	Refr. solids (Undilute)	Pol	Sucrose %	Red Sugars %	Sulph. Ash %	Colour (a*c) 560 mμ	Viscosity in poise at 80% D.S.		Filterability %	Red. Sugar/Ash Ratio	True Purity	Target Purity			True Purity - Target Purity		
									50°C	25°C				Douwes-Dekker	Australian Formula	Mauritian Formula	Douwes-Dekker	Australian	Mauritian
PG	NOV	81.62	84.05	35.84	37.50	11.61	15.18	31.6	74.8	542.2	—	0.76	45.94	42.72	42.79	41.56	3.22	3.15	4.38
ML	JULY	79.45	81.94	31.44	35.66	21.10	10.99	32.9	21.3	213.1	—	1.92	44.88	38.53	35.63	36.92	6.35	9.25	7.96
	SEPT	80.36	83.20	32.48	34.72	17.26	12.95	46.8	30.3	283.7	63	1.33	43.20	40.22	38.46	39.28	2.98	4.74	3.92
	NOV	79.80	81.54	37.92	41.90	13.04	11.94	46.1	9.2	142.8	—	1.09	52.45	41.30	40.00	40.24	11.15	12.45	12.21
UF	JULY	82.67	85.39	34.74	38.34	14.72	16.41	36.9	35.0	337.2	—	0.90	46.38	42.84	41.48	41.00	3.54	4.89	5.38
	SEPT	82.88	84.56	36.40	39.46	13.28	16.87	31.5	22.8	197.0	76	0.79	47.61	43.53	42.49	41.44	4.08	5.12	6.17
	NOV	81.48	84.72	36.44	36.53	13.11	16.03	35.2	—	—	—	0.82	44.83	42.82	42.20	41.32	2.01	2.62	3.51
	JAN	82.28	85.08	34.92	38.62	16.87	14.67	33.5	41.8	398.6	75	1.15	46.94	41.51	39.60	40.00	5.43	7.34	6.94
EM	JULY	84.00	86.90	36.14	39.09	12.38	16.62	63.0	128.3	730.8	—	0.74	46.54	43.30	43.00	41.64	3.24	3.54	4.90
	SEPT	83.37	85.80	33.76	36.42	15.34	14.84	51.7	56.0	450.8	50	1.03	43.68	41.48	40.44	40.48	2.20	3.24	3.20
	NOV	81.19	84.93	36.68	38.65	10.77	15.25	51.7	96.5	777.1	—	0.71	47.60	43.18	43.32	41.76	4.42	4.28	5.84
	JAN	83.03	86.02	33.20	38.72	14.70	14.41	41.6	50.9	366.1	45	1.02	46.63	41.67	40.52	40.52	4.96	6.11	6.11
FX	JULY	82.56	86.40	31.00	34.62	15.23	16.80	38.6	77.8	656.7	—	0.91	41.93	42.44	41.40	40.96	-0.51	0.53	0.97
	SEPT	84.14	85.70	33.48	34.44	15.59	17.01	32.8	43.6	342.3	67	0.92	40.93	42.26	41.31	40.92	-1.33	-0.38	0.01
	NOV	83.15	86.39	33.48	34.08	13.79	16.47	40.4	98.4	797.1	—	0.84	40.99	42.36	42.02	41.24	-1.37	-1.03	-0.25
	JAN	84.71	87.44	29.16	34.45	19.88	15.16	37.9	49.1	415.6	63	1.31	40.67	40.54	38.58	39.36	0.13	2.09	1.31
EN	JULY	78.08	81.32	33.94	35.81	11.43	15.19	38.0	27.6	233.5	—	0.75	45.86	43.06	42.89	41.60	2.80	2.97	4.26
	SEPT	78.89	81.80	37.40	36.96	10.38	15.33	38.2	35.0	298.3	76	0.68	46.85	43.39	43.65	41.88	3.46	3.20	4.97
	NOV	78.94	82.69	39.16	39.00	8.03	16.44	44.3	76.0	594.8	—	0.49	49.39	44.97	46.18	42.64	4.42	3.20	6.75
	JAN	80.35	83.81	35.52	37.27	10.86	16.09	38.7	93.4	924.6	68	0.67	46.38	43.58	43.76	41.92	2.80	2.61	4.46
AK	JULY	83.58	86.62	34.00	35.90	16.20	15.76	34.7	53.3	519.7	—	1.03	42.95	41.75	40.44	40.48	1.20	2.51	2.47
	SEPT	84.42	85.20	34.44	35.07	16.61	15.89	31.7	24.5	182.0	71	1.04	41.54	41.60	40.37	40.44	-0.06	1.17	1.10
	NOV	83.38	86.90	36.84	36.31	12.04	16.83	38.1	86.5	494.4	—	0.71	43.55	43.10	43.32	41.76	0.45	0.23	1.79
	JAN	84.79	87.08	31.24	35.09	18.33	15.00	39.7	58.1	543.8	63	1.22	41.38	40.76	39.13	39.72	0.62	2.25	1.66
DL	SEPT	80.64	82.86	33.24	35.00	16.06	14.63	39.7	35.0	320.3	56	1.10	43.40	41.39	39.93	40.20	2.01	3.47	3.20
	NOV	79.14	82.34	36.04	35.09	9.69	16.05	50.4	60.0	471.2	—	0.60	44.34	43.60	44.62	42.20	0.74	-0.28	2.14
	JAN	79.31	82.59	31.48	34.96	15.79	13.25	46.9	40.5	337.5	48	1.19	44.08	40.79	39.33	39.84	3.29	4.75	4.24
DK	JULY	81.20	83.37	33.92	37.43	14.84	14.55	33.7	44.6	444.2	—	1.02	46.10	41.80	40.52	40.52	4.34	5.58	5.58
	SEPT	82.32	84.30	32.88	35.95	15.28	15.54	31.5	44.7	338.6	63	0.98	43.67	41.95	40.83	40.68	1.72	2.84	2.99
	NOV	82.35	85.54	35.94	36.59	12.67	15.50	41.5	59.0	397.0	—	0.82	44.43	42.47	42.20	41.32	1.96	2.22	3.11
	JAN	83.19	86.34	32.32	35.23	16.57	15.33	36.8	45.1	305.1	44	1.08	42.35	41.42	40.08	40.28	0.93	2.22	2.07
GH	SEPT	77.49	80.00	33.12	35.44	14.93	13.58	33.9	18.2	136.9	58	1.10	45.73	41.43	39.93	40.20	4.30	5.80	5.53
	NOV	81.29	84.12	40.04	38.56	10.94	14.50	37.5	90.5	619.5	—	0.75	47.44	42.65	42.89	41.60	4.79	4.55	5.53
	JAN	81.96	85.09	33.20	37.47	16.08	13.78	39.2	51.4	440.3	50	1.17	45.72	41.03	39.46	39.92	4.69	6.26	5.80
GD	JULY	81.83	84.05	32.80	36.22	13.88	17.15	37.3	33.1	258.0	—	0.81	44.26	43.22	42.30	41.36	1.04	1.96	2.90
	SEPT	83.23	84.06	33.60	37.04	15.19	15.82	31.2	22.9	226.0	57	0.96	44.50	42.15	40.98	40.76	2.35	3.51	3.74
	NOV	83.67	85.74	34.08	37.49	12.07	17.08	39.7	52.3	364.6	—	0.71	44.81	43.41	43.32	41.76	1.40	1.49	3.05
	JAN	82.36	84.70	28.76	34.29	17.97	15.04	37.4	38.7	292.0	50	1.19	41.63	41.01	39.33	39.84	0.62	2.30	1.79
MV	JULY	81.90	84.78	31.20	34.17	16.74	16.10	32.5	46.6	542.4	—	1.04	41.72	41.84	40.37	40.44	-0.12	1.35	1.28
	SEPT	82.88	84.06	33.16	34.37	16.80	15.54	28.0	25.1	280.0	—	1.08	41.47	41.43	40.07	40.28	0.04	1.39	1.19
	NOV	83.83	84.09	34.48	35.40	13.37	16.21	30.9	—	—	—	0.82	42.23	42.37	42.20	41.32	-0.14	0.03	0.91
	JAN	81.78	84.84	31.20	34.30	16.49	15.66	34.6	51.0	441.8	49	1.05	41.94	41.67	40.29	40.40	0.27	1.65	1.54
TS	JULY	79.94	82.37	30.52	33.83	17.66	15.05	29.0	38.4	300.5	—	1.17	42.32	41.29	39.46	39.92	1.03	2.86	2.40
	SEPT	82.32	84.20	31.72	32.74	18.33	14.98	25.9	31.6	274.9	66	1.22	39.77	40.77	39.13	39.72	-1.00	0.64	0.05
	NOV	78.97	82.14	35.16	33.73	12.73	15.68	30.8	50.1	384.7	—	0.81	42.71	42.64	42.30	41.36	0.07	0.41	1.35
	JAN	80.35	83.09	32.44	35.65	16.59	14.10	32.1	57.7	528.3	50	1.18	44.37	41.10	39.39	39.88	3.27	4.98	4.49
JB	JULY	79.98	84.25	30.26	34.54	15.95	13.65	47.3	71.0	659.7	—	1.17	43.19	40.87	39.46	39.92	2.32	2.86	3.27
	SEPT	80.36	83.60	32.04	34.01	14.61	13.58	48.5	56.2	558.5	78	1.07	42.32	40.97	39.46	40.32	1.35	2.86	2.00
	NOV	82.36	86.94	31.12	39.22	14.95	13.80	48.3	120.7	855.8	—	1.08	43.01	40.97	40.07	40.28	2.04	2.94	5.73
UC	JULY	84.14	87.99	33.92	37.98	16.32	13.30	47.7	65.1	573.2	—	1.23	45.14	40.53	39.07	39.68	4.61	6.07	5.46
	SEPT	83.30	86.10	34.00	36.20	15.08	13.30	42.9	55.5	447.1	61	1.13	43.46	40.65	39.72	40.08	2.81	3.73	3.38
	NOV	83.21	86.94	35.16	36.93	13.95	13.52	42.1	71.9	429.5	—	1.03	44.38	41.06	40.44	40.48	3.32	3.94	3.90
IL	JULY	81.06	84.90	30.96	34.85	16.45	14.42	42.3	49.3	430.2	—	1.14	42.99	41.14	39.66	40.04	1.85	3.33	2.95
	SEPT	82.11	84.80	32.68	33.99	15.50	15.12	41.7	55.0	513.1	69	1.02	41.40	41.46	40.52	40.52	-0.06	0.88	0.88
	NOV	80.12	83.52	32.28	32.46	13.46	15.67	39.7	68.5	630.3	—	0.86	40.51	42.17	41.84	41.16	-1.66	-1.33	-0.65
	JAN	82.30	85.69	29.04	34.48	18.33	14.33	41.3	52.2	411.7	61	1.28	41.90	40.59	38.76	39.48	1.31	3.14	2.42
RN	JULY	83.16	85.56	35.64	39.10	13.78	15.61	47.9	44.2	340.1	—	0.88	47.02	42.58	41.65	41.08	4.44	5.36	5.94
	NOV	77.72	80.94	38.60	35.60	8.83	14.91	43.6	49.4	344.5	—	0.59	45.81	43.41	44.75	42.24	2.40	1.06	3.57

TABLE II
Composition of the Molasses Dry Solids

	PG				ML				UF				EM				FX			
	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN
DRY SOLIDS %	—	—	81.62	—	79.45	80.36	79.80	—	82.67	82.88	81.48	82.28	84.00	83.37	81.19	83.03	82.56	85.70	86.39	87.44
SUCROSE % DRY SOLIDS	—	—	45.94	—	44.88	43.21	52.51	—	46.38	47.67	44.83	46.94	46.54	43.68	47.60	46.63	41.93	40.93	40.99	40.67
REDUCING SUGAR % DRY SOLIDS	—	—	14.22	—	26.56	21.48	16.34	—	17.81	16.02	16.09	20.50	14.74	18.40	13.27	17.70	18.45	18.53	16.58	23.47
ASH % DRY SOLIDS	—	—	18.6	—	13.8	16.1	15.0	—	19.9	20.4	19.7	17.8	19.8	17.8	18.8	17.4	20.4	20.2	19.8	17.9
STARCH % DRY SOLIDS	—	—	0.4	—	0.2	0.2	0.2	—	0.1	0.1	0.03	0.2	0.5	0.3	0.4	0.3	0.2	0.2	0.2	0.1
GUMS % DRY SOLIDS	—	—	4.3	—	—	3.0	3.9	—	2.6	2.9	2.9	2.5	4.3	3.8	5.0	3.3	—	3.5	3.6	2.8
WAX % DRY SOLIDS	—	—	—	—	—	0.56	—	—	—	0.29	—	0.40	—	0.77	—	0.77	—	0.54	—	0.65
AC. ACID % DRY SOLIDS	—	—	—	—	—	1.8	—	—	—	1.6	—	1.7	—	1.3	—	1.3	—	2.0	—	1.6
PROTEIN % DRY SOLIDS	—	—	—	—	—	6.3	—	—	—	8.1	—	4.6	—	6.3	—	4.9	—	6.8	—	4.8
	EN				AK				DL				DK				GH			
	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN
DRY SOLIDS %	78.08	78.89	78.94	80.35	83.58	84.42	83.38	84.79	—	80.64	79.14	79.31	81.20	82.32	82.35	83.19	—	77.49	81.29	81.96
SUCROSE % DRY SOLIDS	45.86	46.85	49.40	46.38	42.95	41.54	43.55	41.38	—	43.40	44.34	44.04	46.10	43.67	44.43	42.35	—	45.73	47.44	45.72
REDUCING SUGAR % DRY SOLIDS	14.64	13.16	10.17	13.52	19.38	19.68	14.44	21.62	—	19.92	12.24	19.91	18.28	18.56	15.39	19.92	—	19.27	13.46	19.62
ASH % DRY SOLIDS	19.5	19.4	20.8	20.0	18.9	18.8	20.2	17.7	—	18.1	20.3	16.7	17.9	18.9	18.8	18.4	—	17.5	17.8	16.8
STARCH % DRY SOLIDS	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1	—	0.2	0.2	0.2	0.4	0.2	0.1	0.2	—	0.4	0.3	0.1
GUMS % DRY SOLIDS	2.8	3.1	3.4	2.7	—	3.3	3.6	3.0	—	3.6	4.0	3.4	—	4.0	3.9	3.3	—	4.2	4.2	2.9
WAX % DRY SOLIDS	—	0.13	—	0.28	—	0.38	—	0.72	—	0.64	—	0.98	—	0.80	—	1.11	—	0.54	—	0.95
AC. ACID % DRY SOLIDS	—	2.0	—	2.0	—	1.3	—	1.8	—	2.3	—	1.2	—	2.0	—	2.0	—	1.5	—	1.4
PROTEIN % DRY SOLIDS	—	7.2	—	7.1	—	7.6	—	6.6	—	7.0	—	5.4	—	7.0	—	6.8	—	5.9	—	5.6
	GD				MV				TS				JB				UC			
	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN
DRY SOLIDS %	81.83	83.23	83.67	82.36	81.90	82.88	83.83	81.78	79.94	82.32	78.97	80.35	79.98	80.36	82.36	—	84.14	83.30	83.21	—
SUCROSE % DRY SOLIDS	44.26	44.50	44.81	41.63	41.72	41.47	42.23	41.94	42.32	39.77	42.71	44.37	43.19	42.32	47.62	—	45.14	43.46	44.38	—
REDUCING SUGAR % DRY SOLIDS	16.96	18.25	14.42	21.82	20.44	20.27	15.95	20.16	22.09	22.27	16.12	20.65	19.94	18.18	18.15	—	19.40	18.10	16.76	—
ASH % DRY SOLIDS	21.0	19.0	20.4	18.3	19.7	18.8	19.3	19.2	18.8	18.2	19.9	17.6	17.1	16.9	16.8	—	15.8	16.0	16.3	—
STARCH % DRY SOLIDS	0.5	0.4	0.3	0.3	0.3	0.1	0.3	0.2	0.2	0.1	0.1	0.1	0.3	0.2	0.1	—	0.3	0.2	0.1	—
GUMS % DRY SOLIDS	—	3.7	3.8	3.3	—	3.3	3.5	3.2	—	3.4	3.8	3.1	—	2.8	2.8	—	—	3.0	—	—
WAX % DRY SOLIDS	—	0.72	—	0.84	—	—	—	0.90	—	0.66	—	1.03	—	0.40	—	—	—	0.55	—	—
AC. ACID % DRY SOLIDS	—	1.8	—	2.1	—	—	—	1.8	—	1.9	—	1.6	—	2.2	—	—	—	2.1	—	—
PROTEIN % DRY SOLIDS	—	7.1	—	5.4	—	—	—	5.6	—	6.4	—	—	—	8.1	—	—	—	11.2	—	—
	IL				RN				SZ				UK				AVERAGES			
	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN
DRY SOLIDS %	81.06	82.11	80.12	82.30	83.16	—	77.72	—	80.85	79.52	79.66	—	77.04	—	76.36	73.76	81.34	81.83	81.13	81.76
SUCROSE % DRY SOLIDS	42.99	41.40	40.51	41.90	47.02	—	45.81	—	47.20	46.96	47.59	—	45.26	—	44.89	45.21	44.60	43.54	45.35	43.78
REDUCING SUGAR % DRY SOLIDS	20.29	18.88	16.80	22.27	16.57	—	11.36	—	16.71	16.17	12.21	—	16.60	—	11.26	15.25	18.68	18.60	14.48	19.72
ASH % DRY SOLIDS	17.8	18.4	19.6	17.4	18.8	—	19.2	—	18.6	18.7	18.6	—	18.4	—	21.5	17.8	18.5	18.3	19.0	17.9
STARCH % DRY SOLIDS	0.3	0.3	0.2	0.1	0.4	—	0.3	—	0.3	0.2	0.2	—	0.2	—	0.1	0.1	0.3	0.2	0.2	0.2
GUMS % DRY SOLIDS	—	4.0	3.7	2.8	3.1	—	4.6	—	—	3.8	4.1	—	—	—	4.5	4.2	3.2	3.5	3.8	3.1
WAX % DRY SOLIDS	—	0.40	—	0.57	—	—	—	—	—	0.33	—	—	—	—	—	0.69	—	0.48	—	0.76
AC. ACID % DRY SOLIDS	—	1.6	—	1.8	—	—	—	—	—	1.4	—	—	—	—	—	1.6	—	1.8	—	1.7
PROTEIN % DRY SOLIDS	—	7.1	—	6.9	—	—	—	—	—	5.7	—	—	—	—	—	8.6	—	7.2	—	6.0

TABLE IV
Composition of the Ash of Final Molasses

	ML				UF				EM				FX				EN				
	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	
Sulphated Ash % solids in Molasses	13.83	16.11	14.96	—	19.85	20.35	19.67	17.83	19.79	17.80	18.78	17.36	20.35	20.22	19.81	17.90	19.45	19.43	20.83	20.02	
K ₂ O % Ash	30.66	27.44	23.86	—	28.56	29.63	32.13	30.34	25.57	26.97	26.89	26.67	28.94	27.30	25.44	25.36	25.50	26.09	19.73	22.08	
CaO % Ash	8.10	9.25	12.63	—	7.00	5.80	7.42	7.18	9.55	8.48	8.73	8.81	8.21	7.37	8.13	8.99	12.96	12.76	12.82	13.79	
MgO % Ash	6.43	6.21	5.95	—	7.00	5.65	6.56	6.34	5.61	6.40	6.23	5.99	6.49	5.19	7.17	7.09	6.53	5.87	5.47	5.89	
R ₂ O ₃ % Ash	—	1.74	—	—	—	0.59	—	0.50	—	2.02	—	1.32	—	0.35	—	0.61	—	0.51	—	0.50	
SiO ₂ % Ash	—	1.97	—	—	—	—	—	2.41	—	2.92	—	2.59	—	2.08	—	2.15	—	2.11	—	1.95	
SO ₄ ²⁻ % Ash	—	16.45	—	—	—	15.13	—	13.57	—	15.84	—	11.52	—	14.98	—	13.52	—	17.76	—	16.93	
Cl ⁻ % Ash	11.93	11.81	10.67	—	17.19	16.71	16.81	15.73	15.12	16.03	15.41	15.10	16.70	16.88	16.38	16.06	13.14	12.52	11.96	12.31	
P ₂ O ₅ % Ash	—	1.43	—	—	—	0.85	—	0.89	—	1.65	—	1.61	—	1.30	—	1.34	—	0.57	—	0.57	
	AK				DL				DK				GH				GD				
	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	
	Sulphated Ash % solids in Molasses	18.86	18.82	20.18	17.69	—	18.14	20.28	16.71	17.92	18.87	18.82	18.43	—	17.52	17.84	16.81	20.96	19.01	20.41	18.26
	K ₂ O % Ash	31.07	29.54	29.38	28.66	—	29.71	30.82	26.39	30.19	30.58	26.46	27.40	—	29.79	29.65	27.54	33.40	30.30	31.11	30.39
	CaO % Ash	7.90	8.29	7.93	10.29	—	6.89	8.73	8.20	7.37	9.22	8.61	8.84	—	8.16	8.18	9.46	7.16	8.36	7.40	9.86
	MgO % Ash	7.42	6.69	6.84	6.67	—	6.84	6.41	7.60	7.70	7.47	6.80	6.56	—	6.68	6.56	6.72	6.49	5.73	5.88	6.02
	R ₂ O ₃ % Ash	—	0.21	—	0.68	—	0.72	—	1.08	—	1.01	—	0.70	—	0.91	—	0.83	—	0.89	—	0.88
	SiO ₂ % Ash	—	1.54	—	2.54	—	2.15	—	3.05	—	1.59	—	1.79	—	2.00	—	2.20	—	1.52	—	2.90
	SO ₄ ²⁻ % Ash	—	13.07	—	14.98	—	15.32	—	14.42	—	14.57	—	13.94	—	15.01	—	18.56	—	13.47	—	13.58
	Cl ⁻ % Ash	16.42	16.52	15.21	14.63	—	16.80	16.63	16.23	16.42	16.52	15.21	14.63	—	16.92	16.45	15.82	15.69	16.40	16.19	15.06
P ₂ O ₅ % Ash	—	1.15	—	1.19	—	1.77	—	1.85	—	1.15	—	1.19	—	0.68	—	0.75	—	1.41	—	1.24	
	MV				TS				JB				UC				IL				
	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	
	Sulphated Ash % solids in Molasses	19.66	18.75	19.34	19.15	18.83	18.20	19.86	17.55	17.07	16.90	16.76	—	15.81	15.97	16.25	—	17.79	18.41	19.56	17.41
	K ₂ O % Ash	26.09	—	28.34	25.54	33.08	38.68	29.31	34.02	28.35	33.85	27.50	—	28.84	32.31	31.45	—	30.97	36.01	27.76	35.55
	CaO % Ash	9.31	—	8.01	9.14	6.69	8.13	9.82	8.72	11.07	10.06	11.63	—	8.03	9.70	9.91	—	7.53	7.60	8.84	10.45
	MgO % Ash	7.63	—	6.72	7.36	3.19	6.10	6.39	6.38	5.15	4.17	4.71	—	5.44	6.82	4.80	—	6.58	6.14	5.62	5.92
	R ₂ O ₃ % Ash	—	—	—	0.99	—	0.33	—	0.74	—	1.77	—	—	—	1.31	—	—	—	0.98	—	0.69
	SiO ₂ % Ash	—	—	—	2.51	—	1.98	—	2.05	—	0.71	—	—	—	1.50	—	—	—	1.30	—	2.35
	SO ₄ ²⁻ % Ash	—	—	—	14.83	—	15.82	—	10.12	—	14.67	—	—	—	15.97	—	—	—	12.17	—	13.27
	Cl ⁻ % Ash	15.06	—	15.96	15.55	16.72	17.20	16.75	15.56	13.57	13.01	13.69	—	14.95	15.22	14.70	—	16.71	16.51	16.49	15.41
P ₂ O ₅ % Ash	—	—	—	0.98	—	0.86	—	1.17	—	0.37	—	—	—	0.52	—	—	—	0.47	—	0.68	
	RN				SZ				UK				AVERAGES								
	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	JULY	SEPT	NOV	JAN	
	Sulphated Ash % solids in Molasses	18.77	—	19.18	—	18.61	18.66	18.64	—	18.35	—	21.45	17.84	—	—	—	—	18.49	18.32	19.01	17.92
	K ₂ O % Ash	30.47	—	20.12	—	—	26.58	31.65	—	19.02	—	30.82	39.13	—	—	—	—	28.71	30.32	27.91	29.16
	CaO % Ash	8.95	—	10.37	—	—	8.57	7.99	—	8.34	—	8.90	8.95	—	—	—	—	8.56	8.55	9.22	9.40
	MgO % Ash	7.46	—	7.25	—	—	7.07	7.13	—	8.12	—	5.87	8.00	—	—	—	—	6.73	6.22	6.31	6.66
	R ₂ O ₃ % Ash	—	—	—	—	—	0.91	—	—	—	—	—	1.62	—	—	—	—	—	0.95	—	0.86
	SiO ₂ % Ash	—	—	—	—	—	1.77	—	—	—	—	—	1.85	—	—	—	—	—	1.79	—	2.33
	SO ₄ ²⁻ % Ash	—	—	—	—	—	16.72	—	—	—	—	—	15.02	—	—	—	—	—	15.13	—	14.17
	Cl ⁻ % Ash	15.37	—	16.01	—	16.25	16.48	16.38	—	17.15	—	16.88	20.32	—	—	—	—	15.49	15.70	15.46	15.57
P ₂ O ₅ % Ash	—	—	—	—	—	0.64	—	—	—	—	—	0.92	—	—	—	—	—	1.00	—	1.11	

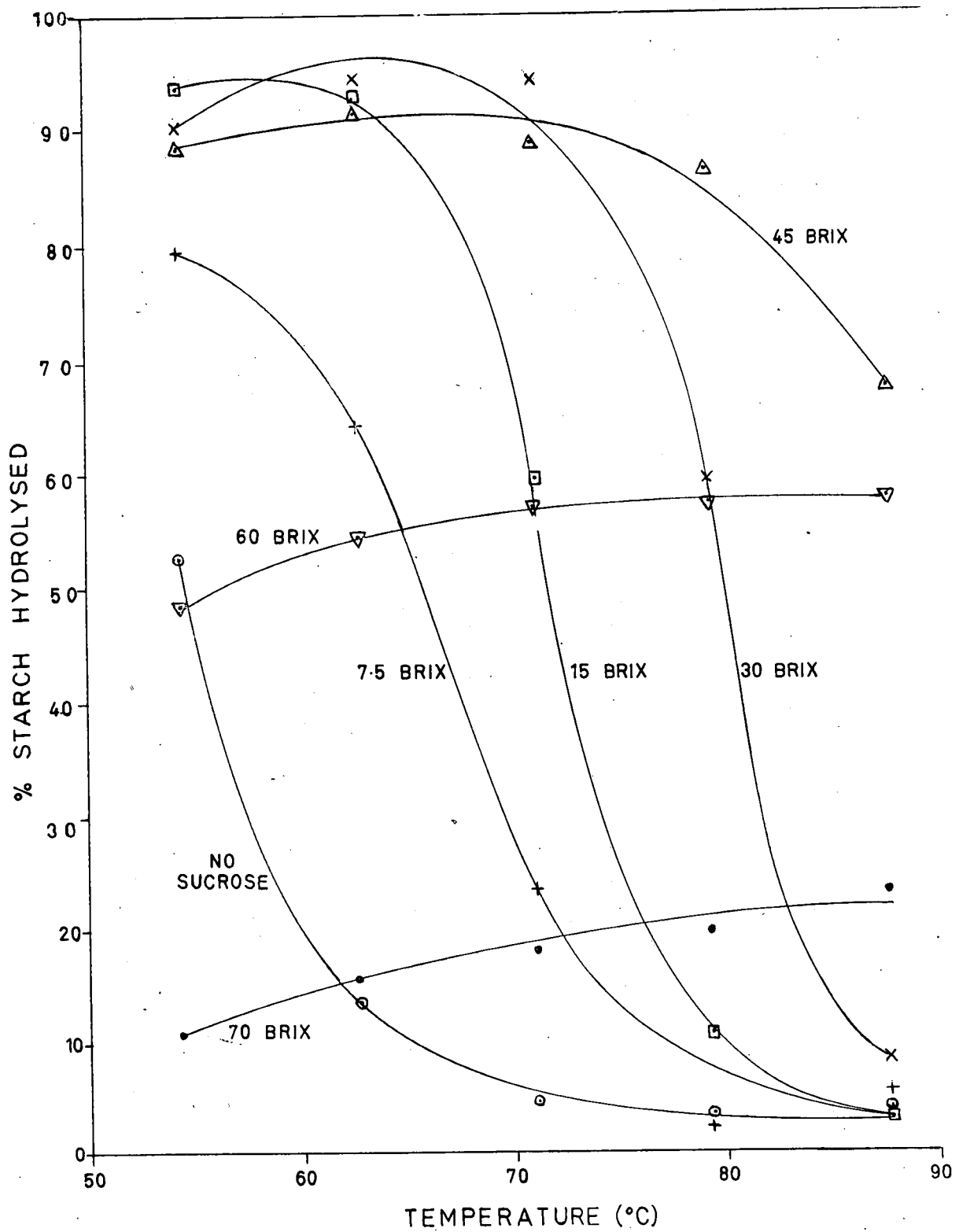


FIGURE 1: Test solution: Sucrose/starch.
 Enzyme dosage: 10 mg CS-250/kg brix.
 Reaction time: 30 minutes.

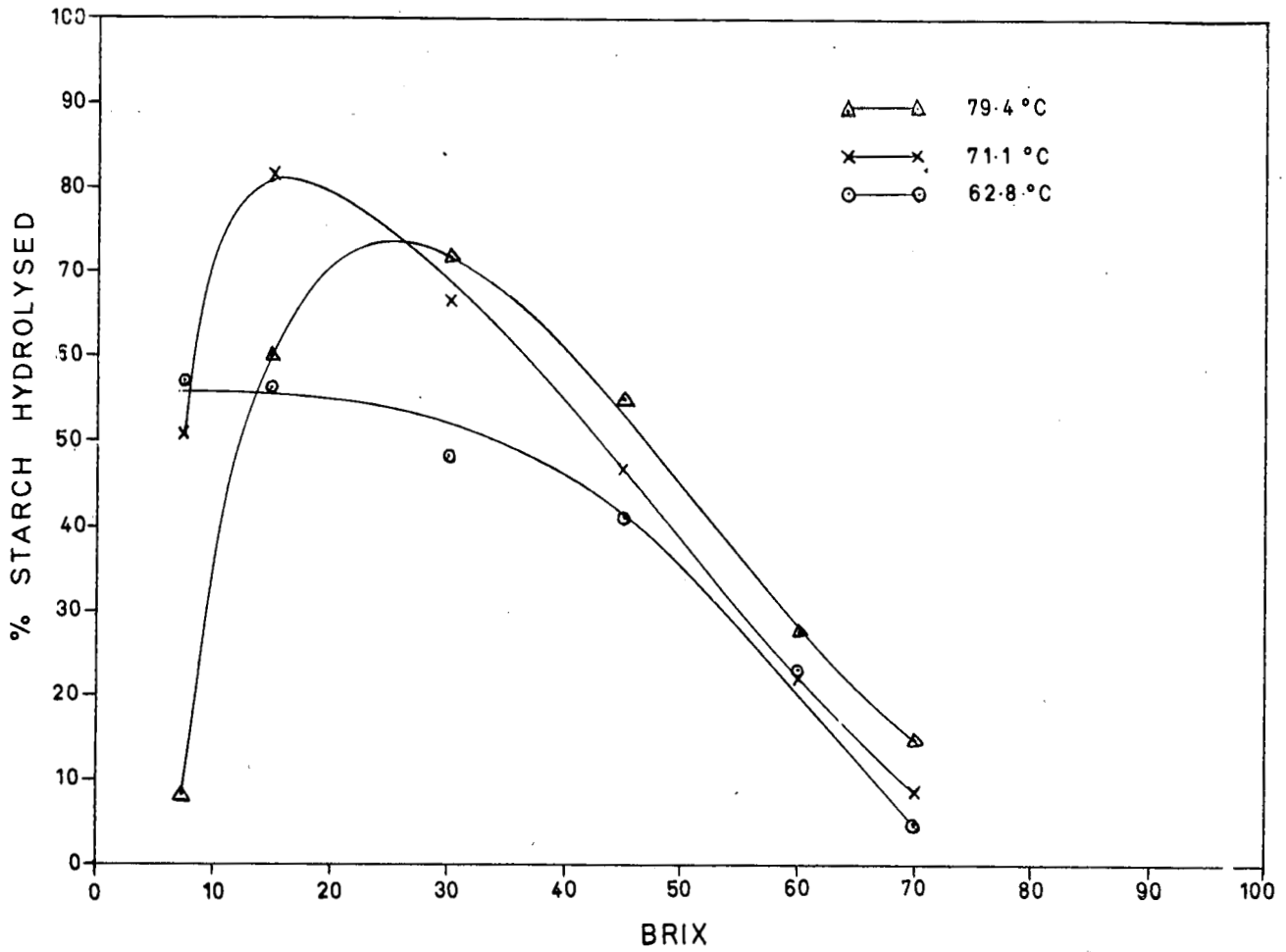


FIGURE 2: Test solution: Syrup.
Enzyme dosage: 10 mg Enzyme A/kg brix.
Reaction time: 30 minutes.

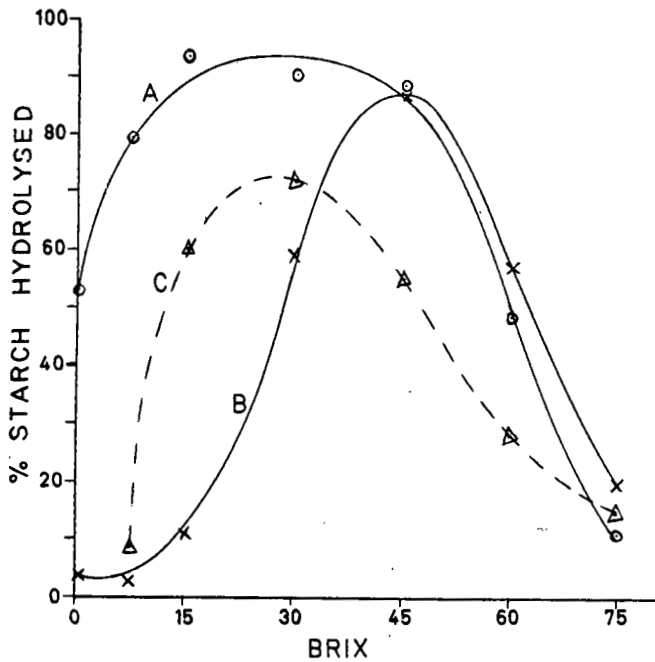


FIGURE 3: Test solution — curves A and B: Sucrose/starch.
Test solution — curve C: Syrup.
Enzyme dosage: 10 mg Enzyme A/kg brix.
Reaction time: 30 minutes.
Temperature — curve A: 54.4°C.
Temperature — curves B and C: 79.4°C.

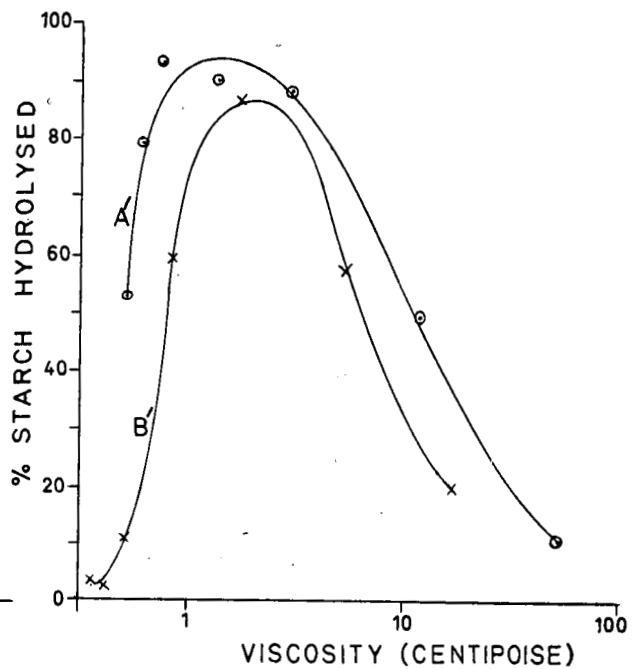


FIGURE 4: Test solution: Sucrose/starch.
Enzyme dosage: 10 mg Enzyme A/kg brix.
Reaction time: 30 minutes.
Temperature — curve A: 54.4°C.
Temperature — curve B: 79.4°C.

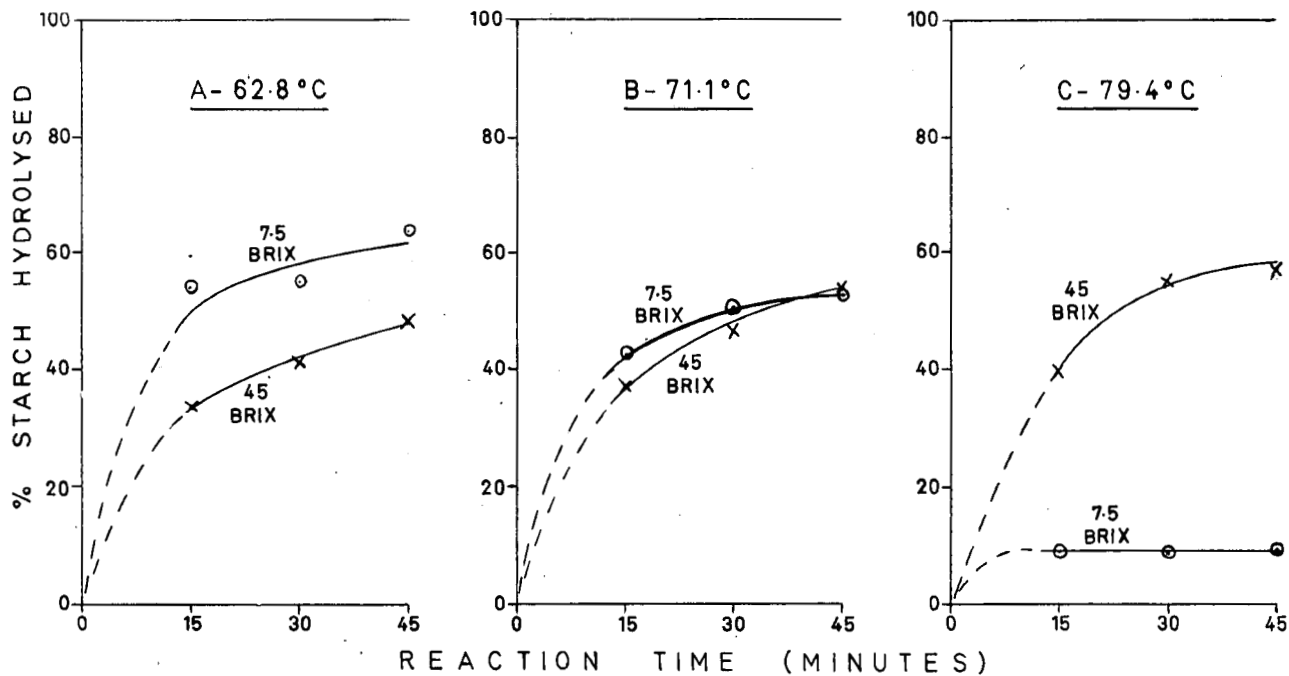


FIGURE 5: Test solution: Syrup.
Enzyme dosage: 10 mg Enzyme A/kg brix.

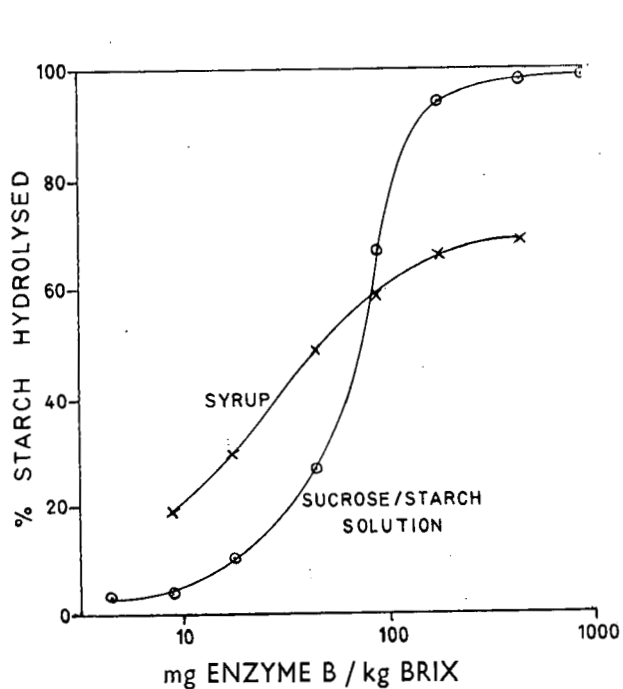


FIGURE 6: Brix: 45.
Temperature: 80°C.
Reaction time: 20 minutes.

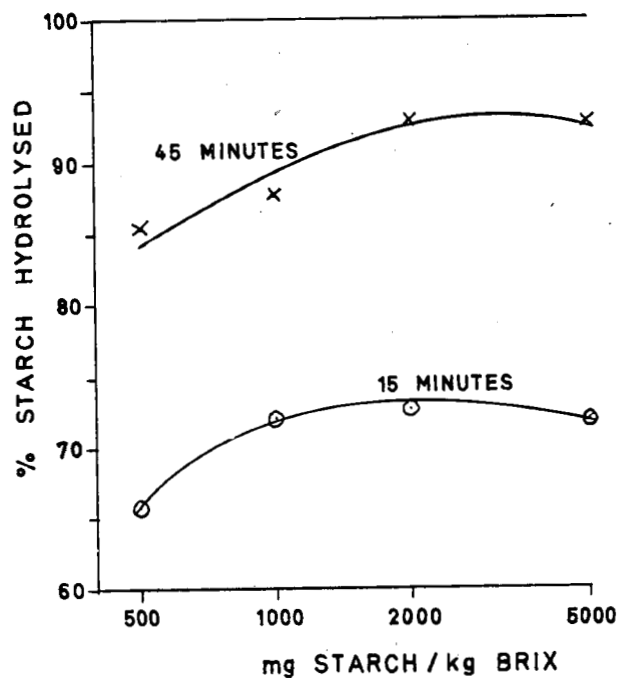


FIGURE 7: Enzyme dosage: 10 mg Enzyme A/kg brix.
Test solutions: Sucrose/starch.
Brix: 45.
Temperature: 71.1°C.