OPTIMUM CONDITIONS OF ENZYMATIC LIQUEFACTION OF STARCHES

BY

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ABSTRACT

A new alpha amylase enzyme (Termamyl 120 L) has been investigated to evaluate the role of this enzyme in starch liquefaction process. The effect of substrate concentration using cassava and corn soluble starches on the enzyme activity indicated that the maximum reaction velocity (V_{max}) was 2.02 mM / l and 1.82 mM / l , respectively .

The Michaelis constant (K_m) was 0.0222mM / l for cassava starch and 0.0321 mM / l for corn starch . The effect of pH on reaction activity of this enzyme indicated that the optimum pH values for (Termamyl 120 L) enzyme was 6.2 in corn soluble starch and 6.7 for cassava starch . The effect of temperature on the enzyme activity declared that the optimum temperature for both types of starch equals 90°C .

The effect of Termamyl 120 L enzyme concentration on the reaction activity was determined , the latter was proceeded with increasing in concentration , but not in proportional .

INTRODUCTION

In the last decade , many scientists stated that cassava(Manihot esculenta) can be used as a raw material for the manufacture of glucose and high fructose syrup in many tropical and subtropical countries. The utilization of this source is particulary interesting for its high yield, one hectare (2.4 feddan) yields 39 -- 45 tons of raw cassava starch corresponding to 10 -- 15 tons of dry starch . Therefore , the introduction of cassava in Egypt with no doubt will be helpful in solving shortage of sucrose, also corn starch can be substituted by this cheaper source to produce glucose and inverted sugar .

There are many disadvantages during acid hydrolysis of starch , Guel (1962) . Consequently , the enzymic degradation is recently of great industrial importance .

Novo Industrials Comp. introduced in (1979) a heat alpa amylase enzyme (over 100°C) which was quite suitable for liquefication process

under the commercial name "Termamyl 120 L " . At this point of temperature (over 100) the risk of retrogradation phenomenon is greatly reduced (Madsen et al. , 1973) and the next saccharification process became easier on cooked starch . This enzyme is a liquid - endo amylase which rapidly hydrolyses 1,4- alpha - glucosidic linkages , at random , in amylose and amylopectin producing dextrins and oligosaacharides .

Rosendal et al.(1979) stated that the stability of the new amylase enzyme " Termamyl 120 L " is greatly depend on : temperature, pH, starch dry substance and salt content . Aschengreen (1979) stated that the optimum pH values ranged between 6.0 and 6.5, added that at low pH values (less than 6.0) and too long a holding time at 95°C might result in the formation of various undesired by - products . However ,Cantarow and Schepartz (1962) reported that amylase in general is not stable below pH 4.5 . He proposed the following conditions for liquefaction of tapioca starch : dry substance 30 % w/w ,pH 6.5 , Ca^{++} content 40 p.p.m., Termamyl dosage 0 .15 % and time at 105°C for 5 min. then at 95°C for 60-- 120 min.

During this work the optimum conditions of alpha amylase enzyme (Termamyl 120 L) were determined in a trial to obtain the most suitable conditions for liquefaction process . The evaluated conditions included : substrate concentration , pH , temperature and enzyme concentration . Also , the chemical and physical properties of cassava starch were determined.

MATERIALS AND METHODS

Materials :

The alpha amylase enzyme (Termamyl 120 L) was supplied by Novo Industria A / S Comp. , Denmark with the following specification , activity equals to 120 K N G / g (One Novo alpha amylase Unit is the amount of enzyme which hydrolyses 5.26 g starch per hour , 37°C at pH 5.6) .E.C 3.2.1.1.alpha 1→ 4—gluconohydrolase .

The soluble starch (corn) was purchased from Starch and Glucose Comp; Cairo , Egypt .

Cassava tubers were obtained from the Agriculture Co-operative Soc; for land reclaimation at El Adlia, Sharkia , Egypt . Starch was prepared from these tubers according to the method reported by Meisel and Jackson (1960)

Physical and chemical properties of cassava and its extracted starch

Crude and total sugar content were determined according to the method reported by Hodge and Davies (1952) .

Reducing and non reducing sugars , crude proten content , crude fibers , acid number , crude fat , moisture and ash content were determined according to the method described by A.O.A.C. (1975) .

Hydrocyanic acid content was determined according to the method reported by Holleman and Atten (1956) .

Density was calculated at 20°C according to the method of Jacobs (1958).

The relative viscosity of 0.25 % (w /w) cassava starch solution was measured according to the method stated by Saad (1985) .

The specific optical rotation was determined by Stanely polarimeter , the $[\alpha]_D^{20}$ was calculated as reported by Willard et al. (1974).

Effect of different parameters on the reaction velocity of alpha amylase enzyme (Termamyl 120 L) ;

1 : effect of substrate concentration

The reaction velocity of alpha amylase (Termamyl 120 L) was tested using different concentration of starch solutions , i.e.0.1 , 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, 1.7, 1.9 and 2.1 % in phosphate buffer (0.1 M / 1 , pH 6.7) . This process was carried out at optimum dilution of alpha amylase enzyme , 90°C for 30 min. The determination of the produced glucose was carried out colorimeterically as described by Nelson (1944) and which was modified by Somogyi (1952) .

2 : Effect of pH on the activity of alpha amylase enzyme (Termamyl 120L)

The activity of alpha amylase enzyme was tested on different pH values i.e. 5.2, 5.7, 6.2, 6.7, 7.2 and 7.7 in phosphate buffer (0.1 mole / 1), using 2.5 ml. of starch (0.5 %)as substrate and 0.5ml. diluted enzyme . The incubation period was 30 min. at 90°C and the reaction was stopped by adding o.5 ml. of sodium hydroxide soln. (2mole / 1) to all the batches . The determination of the resulted glucose was carried out colorimeterically as mentioned before .

3 : Effect of temperature on the activity of alpha amylase enzyme (Termamyl 120 1)

The activity of alpha amylase enzyme (Termamyl 120 L) was tested at different temperatures i.e., 50, 60, 80, 90 and 100 , using 2.5 ml.

of starch soln. (0.5 %) in phosphate buffer (0.1 mole / l), pH (6.7) as substrate . The incubation period was 30 min. The reaction was stopped as previously mentioned and determined colormeterically.

4 : Effect of alpha amylase enzyme (termamyl 120 L) concentration on reaction activity .

The enzymeaactivity was measured according to the method described by Plummer (1978) .

RESULTS AND DISCUSSION

1 : Physical and chemical properties of cassava

Physical and chemical properties of cassava tubers and its starch are shown in table (1) . The yield of starch from cassava tubers amounted to (79.8 %) of its total solids , consequently cassava is considered as an important source for starch production . This result is in good agreement with the results obtained by Foda (1966), Topias et al. (1978) and El- Fieshawy (1986) .

Hydrocyanic content of cassava starch was found to be less than the amount mentioned by Pipeira et al. (1960), Foda (1966) and EL - Fieshawy (1986) . Therefore , this type of cassava starch could be classified as sweet variety .

Fiber , fat , ash , reducing and non reducing sugars , acid number , moisture and protein content were in concident with the reviewed literature .

The values of density of cassava tubers and extracted starch are similar to those reported by Radley (1953) and Foda (1966). The intrinsic viscosity of cassava starch (0.85) is less than those reported by Ciacco and D'Appolonia (1978); (1.23--1.35) . Such decrease in intrinsic viscosity might attributed to the low solubility of cassava starch , in addition to the irregular dispersion of starch molecules in the solution , Tomas (1983) .

The specific optical rotation of the under study starch using water as solvent (181.6°) is less than the values of potato and legume starch which ranged from 200° to 204° using aqueous calcium chloride as mentioned by Whistler and BeMiller (1965) . The lower value of cassava starch might be due to the the starch granules were

not completely soluble in distilled water . Therefore, it could be recommended that the starch should be dissolved in aqueous calcium chloride solution to give reliable measurements (Whistler and BeMiller , 1965) .

Table(1)Physical and chemical properties of cassava and its extracted starch*.

Component	Cassava tuber		Extracted starch
	Peel	Flesh	
Starch %	——	79.8	83.8
Hydrocyanic acid, mg/kg	10.192	7.941	4.19
Total sugar, %	——	7.200	4.23
Reducing sugar, %	——	4.480	3.60
Non-reducing sugar, %	——	2.720	0.625
Moisture , %	——	9.450	11.470
Viscosity ,	——	——	0.850
Density , g /cm^3	——	1.7513	1.6129
Optical rotation $(\alpha)_D^{20}$	——	——	181.600°
Acid number	——	3.980	34.100
Crude protein	——	6.369	3.396
Fat, %	——	0.287	0.388
Fiber, %	——	3.760	1.080
Ash, %	2.970	4.370	3.490

* These values were calculated on the dry weight basis.

2 : Effect of different parameters on reaction velocity and activity of alpha amylase enzyme (Termamyl 120 L)

2 : 1 : Effect of substrate concentration

This experiment was carried out under constant temperature 90°C for 30 min. and pH 6.7 . The results obtained are shown in table (2) and figure (1, a) . An increase of substrate will result at first in a very rapid rise in reaction velocity rate (one first order reaction) . As the substrate concentration was increased the rate of the reaction slow decrease until the reaction velocity was almost constant at concentration of 2.1 % (1.29×10^{-4} M) for both cassava and soluble starches . The maximum reaction velocity (V_{max}) was higher in cassava starch i.e., 2.02 mM/l than with soluble starch i.e., 1.82 mM / l .

This observation might be attributed to the constitution of differentiation of soluble starch (from maize) and cassava starch , Howling (1980) stated that retrogradation phenomenon has more chance to occur in the case of soluble starch owing to its higher content of amylose . The associated molecules resulted from retrogradation lead to reduce the maximum reaction velocity as noticed in the case of soluble starch .

The Michaelis constant (K_m) of alpha amylase (Termamyl 120 L) is shown in Fig. (1, b) which equals (0.0321 mM / l) for soluble starch and (0.0222 mM / l) for cassava starch. Also ,another method was applied in determining the Michaelis constant i.e. Linweaver and Burk (1954) technique , these results are shown in Fig. (1, c)

2 : 2 : Effect of pH

The effect of pH on reaction activity of alpha amylase enzyme (Termamyl 120 L) was tested at different pH values . Six solutions of cassava starch and soluble starch were adjusted to pH values of 5.2 , 5.7 , 6.2 , 6.7 , 7.2 and 7.7 , table (3) and Fig (2) show that the maximum activity of alpha amylase enzyme on cassava starch is 52.2 umM /l /min. at pH 6.7 which is higher than that of soluble starch 42.8 umM / l / min. at pH 6.2 , the activity falls down on either side of the optimum . Such variation might be attributed to the difference in the lattice form or to the difference in constituents of amylose

Table(2)Effect of substrate concentration on the reaction activity of alpha amylase (Termamyl 120L)

Starch concentration			Soluble starch			Cassava starch		
%	$M \times 10^{-4}$ (s)	$1/s$ ($\times 10^{-4}$)	obtained D-glucose mM/L	(v)	1/v x 10	obtained D-glucose mM/L	(v)	1/v x 10
1.0	6.2	16.13	0.386	0.285	0.351	0.778	0.512	0.195
0.3	18.5	5.41	0.514	0.659	0.152	1.208	1.003	0.100
0.5	30.9	3.24	0.972	0.896	0.112	1.295	1.244	0.080
0.7	43.2	2.31	1.120	1.058	0.095	1.338	1.385	0.072
0.9	55.6	1.79	1.250	1.174	0.085	1.398	1.480	0.068
1.1	67.9	1.47	1.424	1.268	0.079	1.442	1.546	0.065
1.3	80.2	1.25	1.562	1.339	0.075	1.528	1.600	0.063
1.5	92.6	1.08	1.701	1.397	0.072	1.597	1.635	0.061
1.7	104.9	0.95	1.840	1.444	0.069	1.667	1.675	0.059
1.9	117.3	0.85	1.922	1.485	0.067	2.021	1.700	0.058
2.1	129.6	0.77	1.830	1.518	0.066	1.944	1.712	0.058

and amylopectin of starch molecule in cassava starch and soluble starch. Such data are in agreement with those stated by Aschengreen et al.(1979) and Novo (1973) .

Table(3) Effect of pH on activity of alpha amylase enzyme(Termamyl (120L)

pH	Soluble starch		Cassava starch	
	Obtained D glucose µmM/1	Reaction activity µmM/1/min	Obtained D glucose µmM/1	Reaction activity µmM/1/min
5.2	833.3	27.8	1041.3	34.7
5.7	1041.6	34.7	1215.2	40.5
6.2	1284.7	42.8	1354.1	45.1
6.7	1180.5	38.4	1566.6	52.2
7.2	1080.0	36.0	1393.0	49.8
7.7	1041.6	34.7	1423.5	47.5

2 : 3 : Effect of temperature

The effect of temperature on the activity of alpha amylase enzyme was accomplished at different temperatures i.e. 50 , 60 , 80 , 90 and 100 for 30 min., pH 6.7 and 0.5 % of starch solutions in phosphate buffer , the results are shown in table (4) and Fig. (3) . The enzyme activity was accelerated as the temperature increased until 90°C for both cassava starch and soluble starch and then fall down , giving an apparent optimum temperature . The maximum reaction activity for soluble starch was 145.83 u M / 1 /min. which is higher than of cassava starch i.e. 104.16 u M / 1 /min. The optimum temperature is in line with those obtained before by Rosendal et al.(1979) and Aschengreen (1979) .

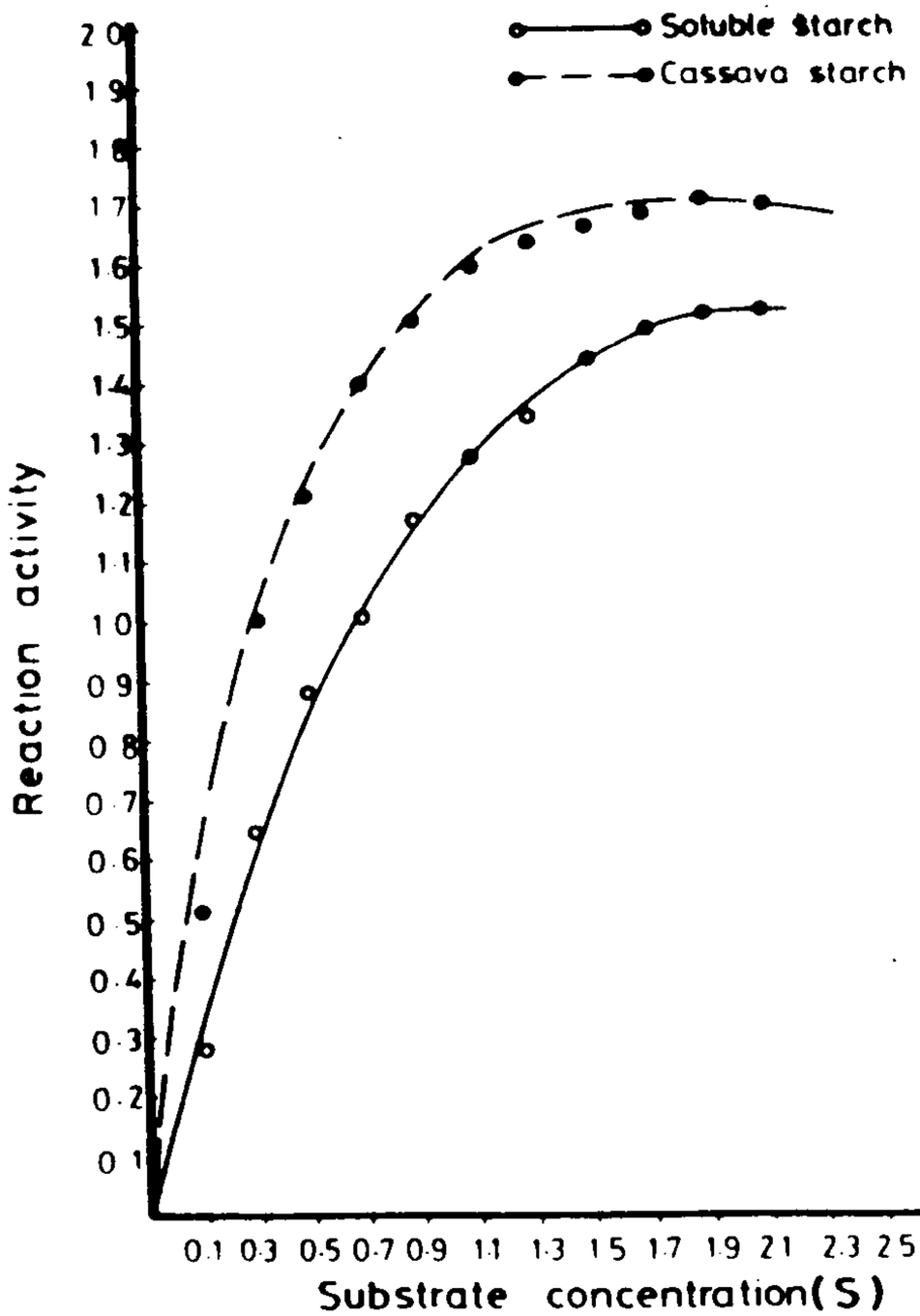

Fig.(1, a) Relationship between substrate concentration and reaction velocity of α-amylase enzyme

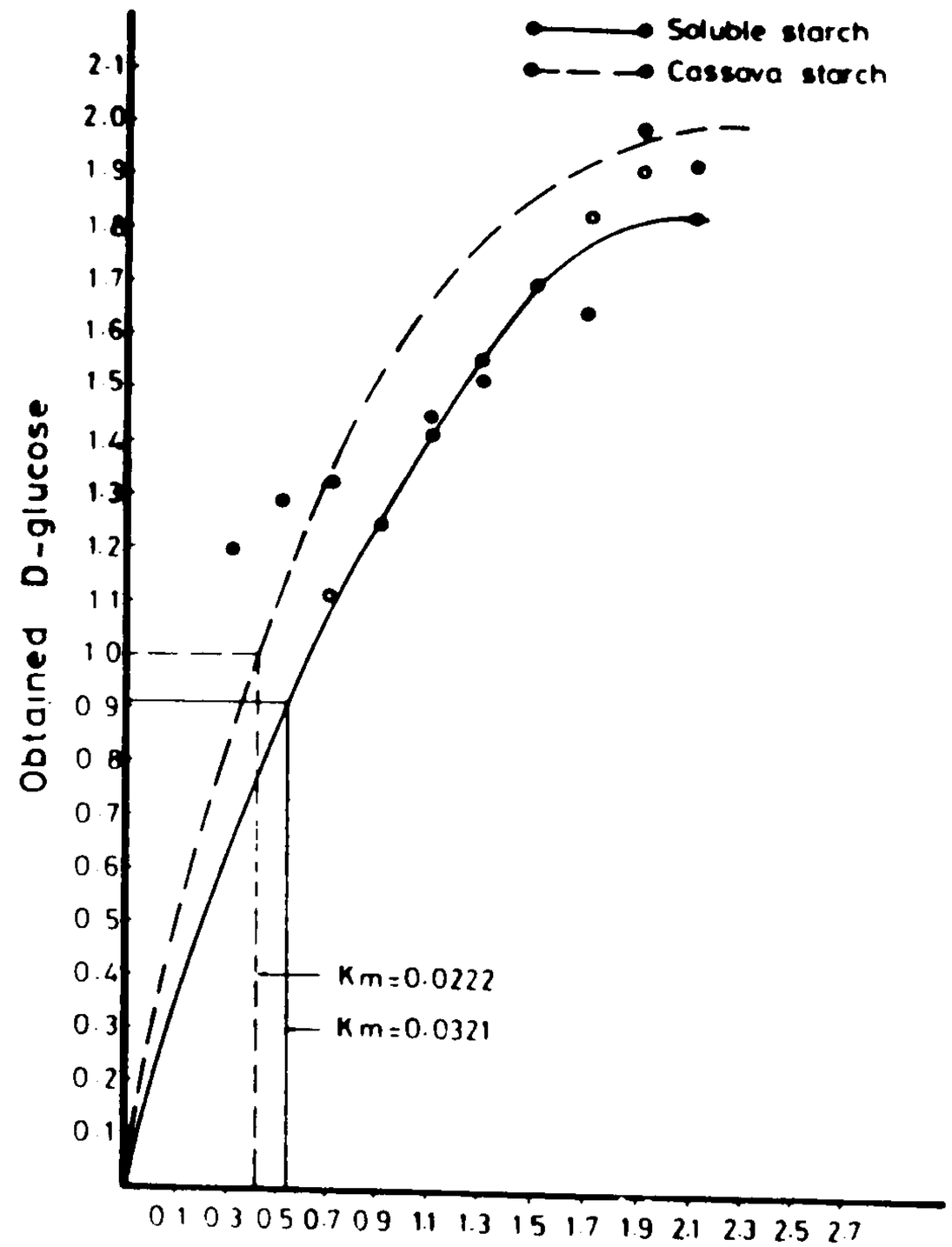

Fig (1, b) Relationship between substrate concentration and obtained D-glucose of α-amylase enzyme

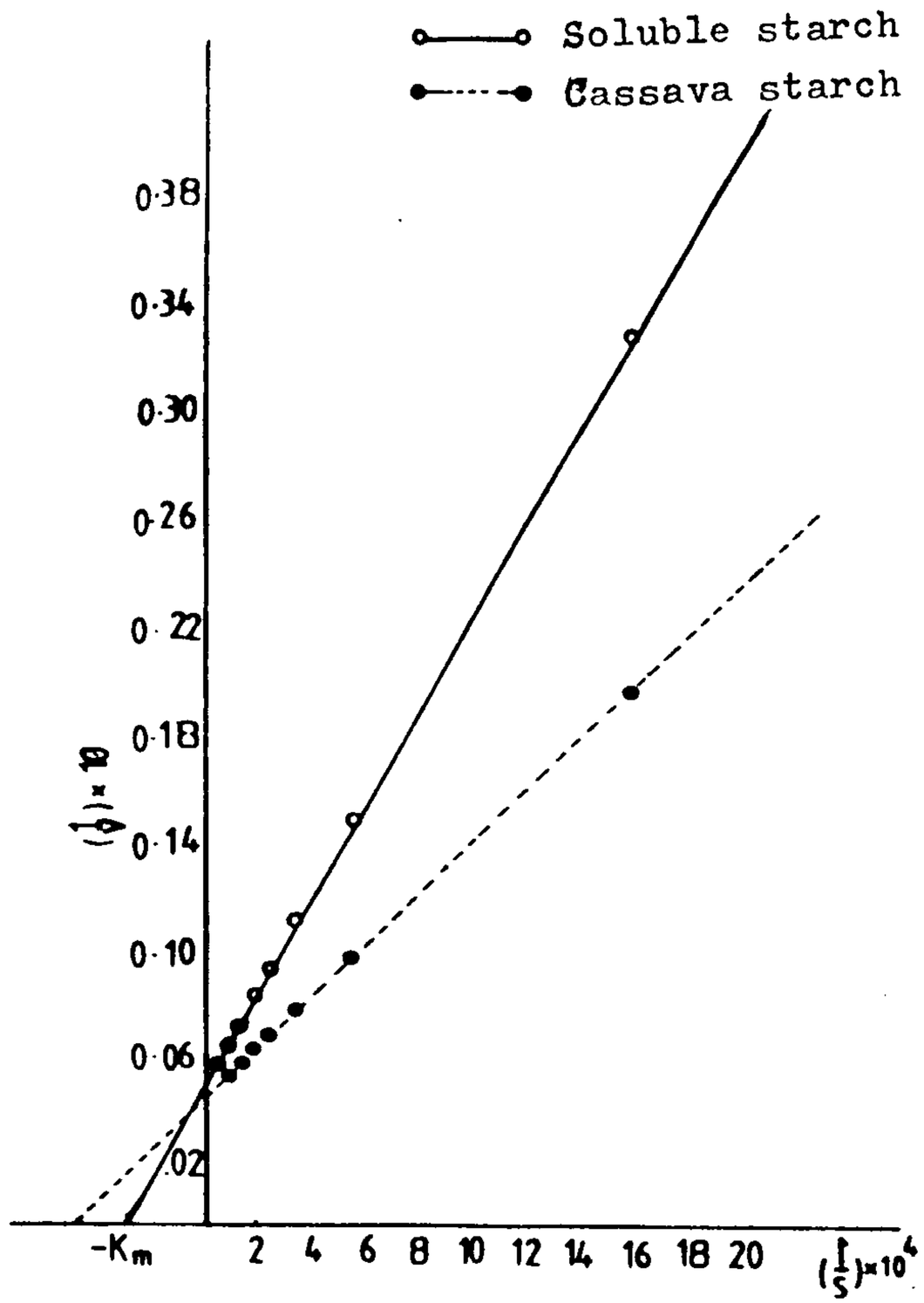

Fig. (1,c) :Effect of substrate concentration on the reaction velocity of α-amylase enzyme (Termamyl)

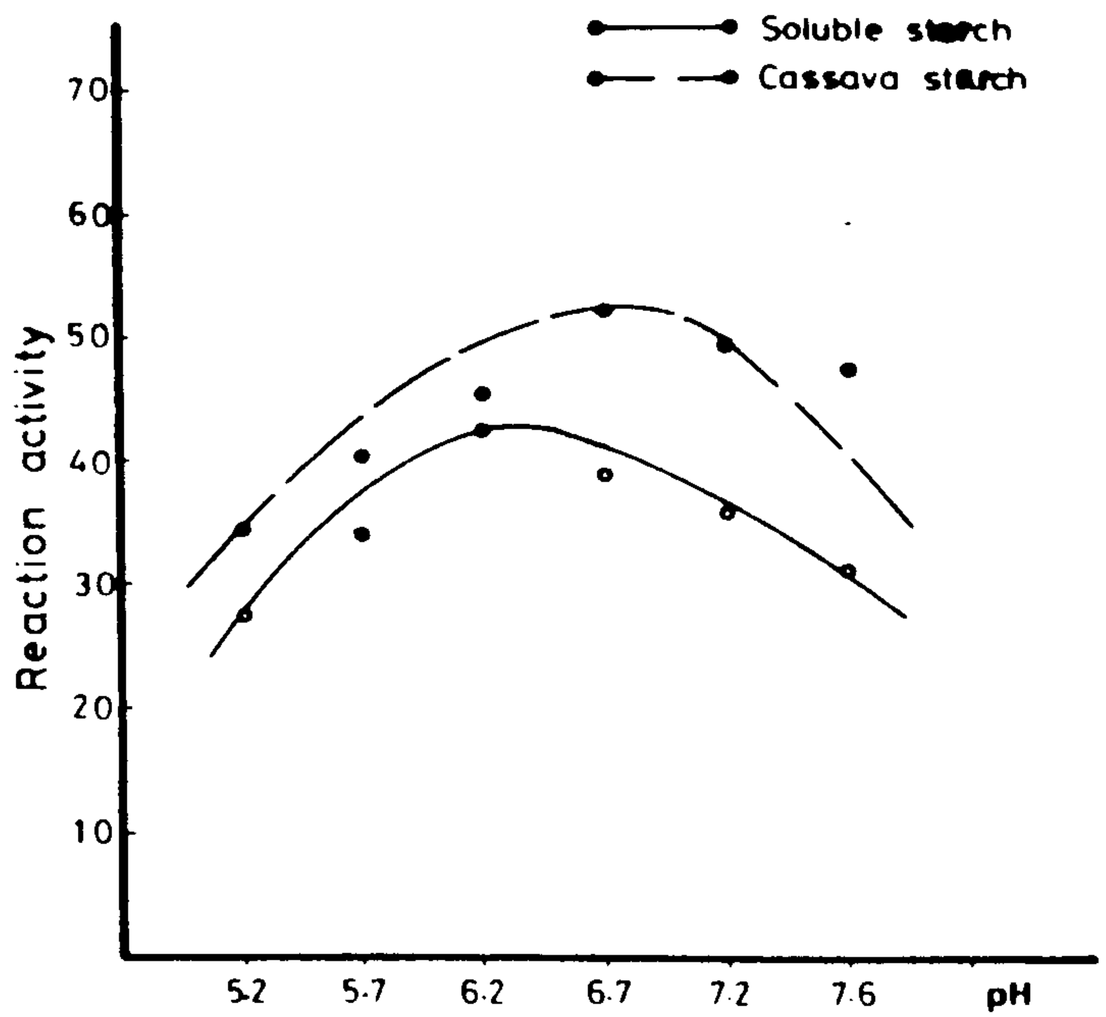

Fig.(2) Effect of pH on the reaction activity of α-amylase enzyme (Termamyl)

Table(4):Effect of temperature on the activity of alpha amylase enzyme(Termamyl 120 L)

Temper. C°	Soluble starch		Cassava starch	
	Obtained D-glucose µmM/1	Reaction activity µmM/1/min	Obtained D glucose µmM/1	Reaction activity µmM/1/min.
50	1055.5	35.18	222.20	7.41
60	1250.0	41.67	750.00	25.00
80	1319.4	43.98	902.70	30.09
90	1458,3	48,61	1041.60	34.72
100	1111.1	37.04	694.40	23.15

2 : 4 : Effect of enzyme concentration on reaction activity

The effect of alpha amylase enzyme concentration on reaction activity in table (5) and Fig. (4) . In general , the velocity of catalyzed reaction is proportional to the concentration of catalyst .

Table(5):Effect of enzyme concentration on the activity of alpha amylase enzyme(Termamyl 120L)

Enzyme concentration I.U / 1 ml	Obtained D-glucose µmM/1	Reaction activity µmM/1/min
0·12	61·72	3·09
0.24	69.44	3.47
0.48	75.62	3.78
1·20	77·16	3.86
2.40	84.88	4.24
4.80	92.59	4.63

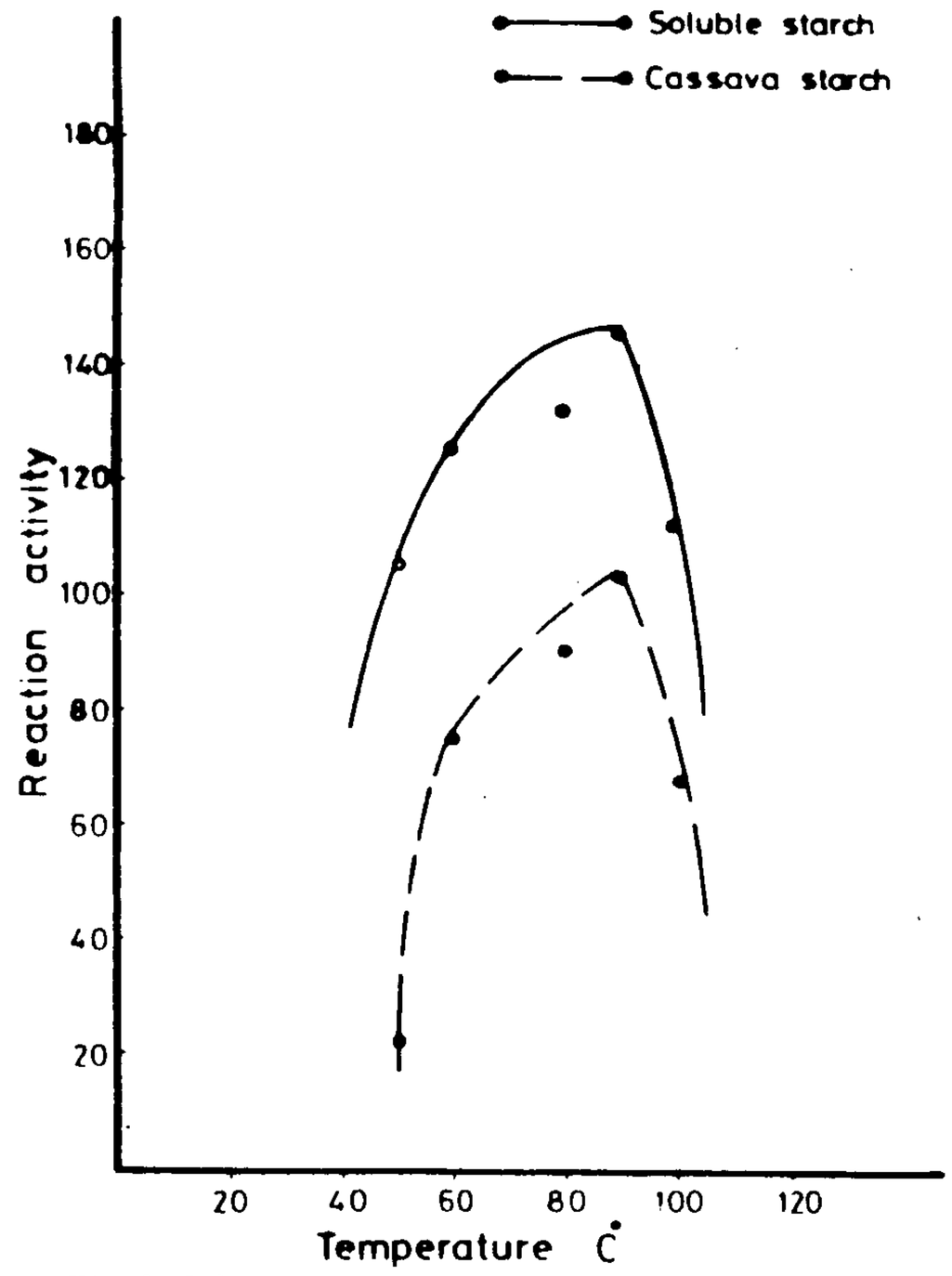

Fig.(3) Effect of temperature on the activity of α- amylase enzyme(Termamyl120 L)

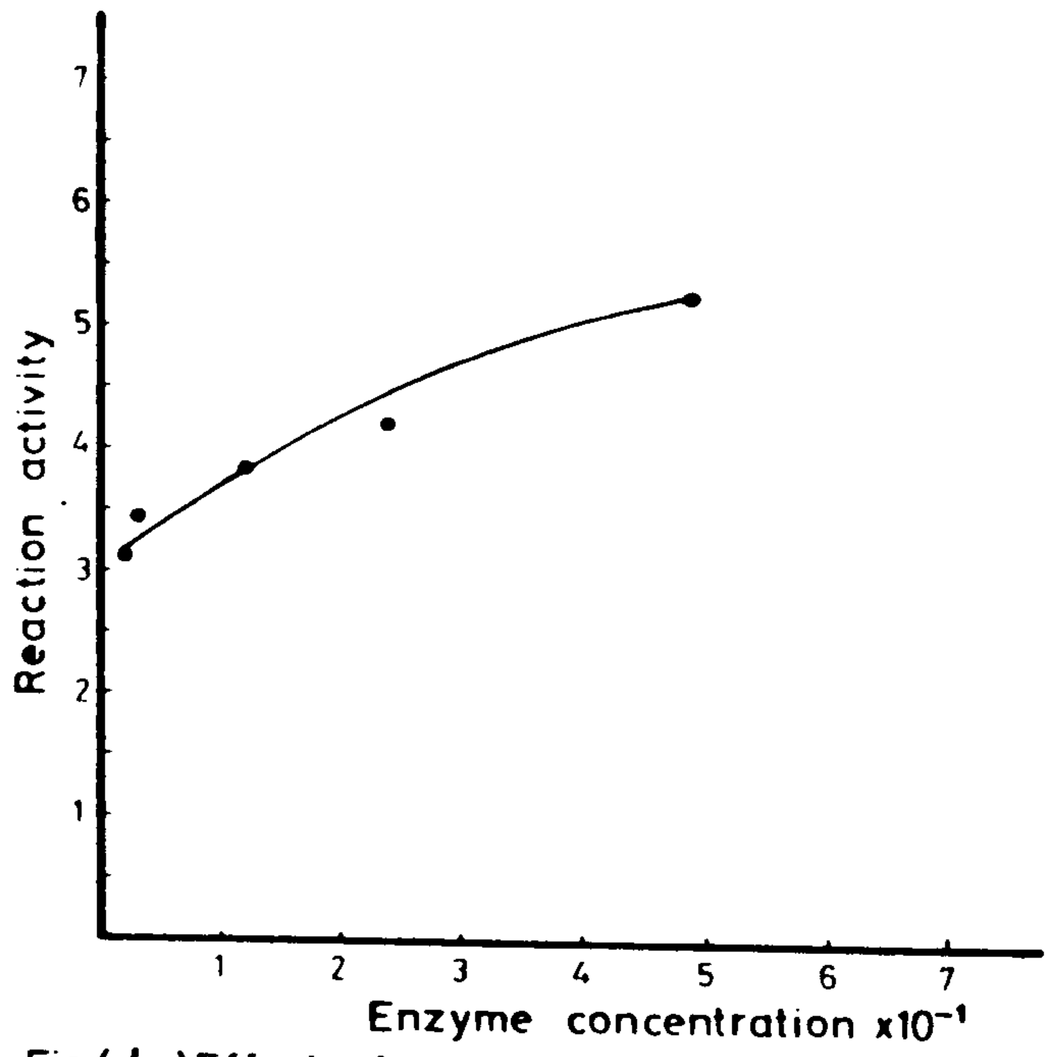

Fig.(4)Effect of enzyme concentration on the reaction activity of α-amylase enzyme (Termamyl 120 L)

The last figure of alpha amylase indicated that this generalization holds true to some extent for this enzymatic reaction . The measured reaction activity was proceeded with increasing enzyme concentration , but not in a proportional way , i.e. the concentration was increased from 0.12 to 4.8 as I.u. /1 ml; but the activity was only increased from 3.09 to 4.63 uM / 1 / min. However, under such condition, e.g high concentration of enzyme , the reaction rate is dependent on substrate concentration , first order reaction .

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الملخص العربى

" الظروف المثلى لتسييل النشا انزيميا "

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أجريت هذه الدراسة لتقييم انزيم الالفا اميليز (الثابت على درجة الحـرارة العالية) الذى تم الحصول عليه من شركة نوفو Novo الدانماركية تحت اسم تجـارى (Termamyl - 120 L) وذلك بهدف استخدامه فى عملية تسييل النشا ٠

وقد درس تأثير تركيز مادة التفاعل على السلوك الحركى لهذا الانزيـــــم واستخدم فى تلك الدراسة نشا الكسافا الذى تم استخلاصه من درنات نبات الكسافـــا وكذلك نشا الذرة الذائب الذى تم الحصول عليه من شركة النشا والجلوكوز بمصـــر وقد تبين من النتائج أن السرعة القصوى للانزيم (٢,٠٢ ملليمول / لتر) فى حالـــة استخدام نشا الكسافا (١,٨٢ ملليمول / لتر) بالنسبة للنشا الذائب وقد وجـــد أن ثابت ميكاليس يساوى (٠٢٢٢, ملليمول / لتر) فى حالة نشا الكسافا أما نشـــا الذرة فكانت قيمته تساوى (٠٣٢١, ملليمول / لتر) ٠درس تأثير درجة الحموضـــة (pH) على نشاط تلك الانزيم ووجد أن الحموضة المثلى للانزيم تساوى ٦,٧ عنـــد استخدام نشا الكسافا كمادة للتفاعل بينما تساوى ٦,٢ فى حالة استخدام النشـــا الذائب ٠ وقد درس أيضا تأثير درجة الحرارة على نشاط الانزيم وتبين أن درجـــة الحرارة المثلى لانزيم الالفا اميليز تساوى ٩٠°م لكلا من مصدرى النشا ٠ وقـــد أوضحت الدراسة أيضا أنه بزيادة تركيز الانزيم يزداد معدل النشاط الانزيمى ولكـــن هذه الزيادة غير منتظمة ٠