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Some factors affecting the haemagglutinin of soybean

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With 1 figure and 3 tables

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Legumes contain haemagglutinins that have the ability to agglutinate the blood cells in various species of animals, and they are destroyed by heat (1) or by autoclaving (2). Inactivation of soybean haemagglutinin by heat is pH-dependent, and at pH 6.7 was the maximum stability towards thermal inactivation (3). The glycoprotein nature of haemagglutinin was established by isolating glucosamine and mannose from haemagglutinin (4). Haemagglutinins were called phytoagglutins or lictins that decrease the food intake and utilization of raw soybean (5).

In this paper we are interested in the haemagglutinin of soybean meal. The effect of temperature on haemagglutinin reaction rates was investigated and the activation energies were calculated.

Experimental

Materials

Samples of soybean seeds variety Rabel, produced in experimental plots of the soybean Research Section of the Ministry of Agriculture, Cairo, Egypt, were air-dried and craked. The flakes were treated with hexane and the defatted meal was ground in a Wiley mill.

Trypsin enzyme was kindly supplied from *Hopkins* and *William* preparation, Chadwell Health, England. Soluble casein and Tris buffer were kindly supplied from B.D.H. Company, England.

Methods

Defatted soybean meak was extracted with water according to the method of *Sambeth* et al. (11). The water extract (fraction 1) was adjusted to pH 4.4, centrifuged, and the acidified supernatant (whey protein, fraction 2) was adjusted to

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pH 8.0 and centrifuged, to give alkaline supernatant (fraction 3). Part of the alkaline supernatant was treated with acetone and centrifuged to give supernatant (fraction 4 A), and precipitate (fraction 4 B). The second part of the alkaline supernatant was treated with saturated ammonium sulphate and centrifuged to give supernatant (fraction 5 A), and precipitate (fraction 5 B).

Haemagglutinin activity of soybean was measured according to the method of *Liener* and *Hill* (2), using fresh red blood cells from rabbit whole blood. One haemagglutinin unit (HU) is the amount of haemagglutinin producing positive evidence of agglutination under the specified conditions.

Determination of carbohydrate content: – The carbohydrate content of soybean were hydrolyzed according to A.O.A.C. method (6). The sugars produced in the hydrolyzate were determined by the phenol sulphuric acid method (7). The results were calculated from standard curve of glucose within concentration 10–70 γ/ml determined under the same condition. Maximum colour developed with 40 minutes and was measured by Carl Zeiss Jena spectrophotometer at 480 mμ.

Protein content of soybean extracts was determined with the semi-micro *Kjeldahl* method (8) using copper sulphate as catalyst. The protein content of column eluates was determined using the modified *Folin* method (9). Maximum colour developed within 45 minutes and was measured by Zeiss Jena spectrophotometer at 750 mμ. The results were calculated from standard curve of bovine serum albumin with concentration 10–100 γ protein per 1 ml.

Results and discussion

1. Extraction of soybean haemagglutinin

Haemagglutinin activity of water extract of unheated defatted soybean, and the various protein preparations from this extract are shown in table 1. The haemagglutinin activity in the water extract was 7137 HU unit per 1 g raw soybean meal, which showed 49 180 HU per 1 g protein (fraction 1). Concerning the carbohydrate content of raw soybean extracts during the progressive steps of protein purification, it is clear from table 1,

Table 1. The haemagglutinin activity of soybean extracts.

Purification step	H U per 1 g soybean	H U per 1 g protein	Carbohydrate g/100 g soybean
Water extract (Fraction 1)	7137	49 180	6.34
Supernatant of pH 4.4 (Fraction 2)	525	14 430	6.27
Supernatant of pH 8.0 (Fraction 3)	525	16 310	4.79
Acetone supernatant of fraction 3 (Fraction 4)	0	0	1.13
Acetone precipitate of fraction 3 (Fraction of 4B)	9	4 880	0.040
(NH_4) SO_4 supernatant of fraction 3 (Fraction 5A)	0	0	1.17
(NH_4) SO_4 precipitate of fraction 3 (Fraction 5B)	17.8	5 985	0.041

HU = Haemagglutinin unit

Table 2. Chromatographic pattern for the elution of haemagglutinin on DEAE-cellulose column.

Chromatography on DEAE-cellulose	mg protein/10 ml fraction	HU per 1 mg protein
Peak 1	0.542	1221.37
Peak 2	0.465	688.17
Peak 3	0.366	872.88
Peak 4	0.495	80.80
Peak 5	0.740	0

that water extract of raw soybean contained 6.34 g carbohydrate per 100 g soybean meal. After adjusting the pH-value of the water extract to pH 4.4 its supernatant contained most of the carbohydrate content (6.27 g/100 g soybean meal), while noticeable decrease in the haemagglutinin activity was found (fraction 2). By raising the pH-value of the supernatant of pH 4.4 (fraction 2) to pH 8.0 a noticeable decrease in the carbohydrate content was found (4.79 g/100 g soybean meal, fraction 3). The relation between the quality of the haemagglutinin and the carbohydrate content that precipitated with the acetone and ammonium sulphate fractions may be due to the participation of carbohydrate with protein as glycoprotein which positively gave the haemagglutinin test (fractions 4 A, 4 B, 5 A, 5 B). *Wolf* et al. (10) fractionated soybean whey on hydroxy-apatite columns and came to this difficulty. They reported that all soybean protein preparations showed a positive test for carbohydrates with phenol sulphuric acid, but it was not well established whether the carbohydrates were contaminants or integral parts of the protein molecules as glycoprotein whereas *Lis* et al. (4) reported that haemagglutinin to be a glycoprotein.

The chromatographic pattern for the elution of haemagglutinating factor of acetone precipitate on DEAE cellulose column is shown in table 2. Under the experimental conditions, the first four peaks eluted after DEAE-cellulose chromatography of soybean showed the presence of haemagglutinin. About 40% of the total haemagglutinin were present in the first protein peak (38.30%). *Sambeth* et al. (11) determined the haemag-

Table 3. Effect of different temperatures on the percentage remaining activity of defatted soybean haemagglutinin.

Duration of treatment (in minutes)	% Remaining activity				
	60	65	70	75 °C	100 °C
0	100	100	100	100	100
5	–	–	–	–	0
10	–	–	–	–	0
15	47.13	46.59	23.17	–	0
20	–	–	–	–	0
30	41.72	23.91	24.07	11.14	–
45	22.39	24.48	21.02	10.63	–
60	22.14	22.63	11.97	10.10	–

glutinin activity of soybean after chromatography on DEAE-cellulose column. Four peaks with maximum haemagglutinin activity were detected in 1st peak. Only 16% of the total haemagglutinin activity were present in the three last protein peaks. In a recent study of *Catsimpoolas* et al., (12) four different forms of haemagglutinin were separated by isoelectric focusing according to their points which ranged between pH 5.85 and 6.2.

Effect of temperature on the soybean haemagglutinin:

The kinetic of heat inactivation of soybean haemagglutinin was studied using water extract of raw defatted material (soybean meal). The percentage of activity remaining of the soybean haemagglutinin units after exposure to different temperatures for different intervals was shown in table 3. Haemagglutinin activity was easily affected by the mild heat treatment and was destroyed completely after five minutes at 100 °C.

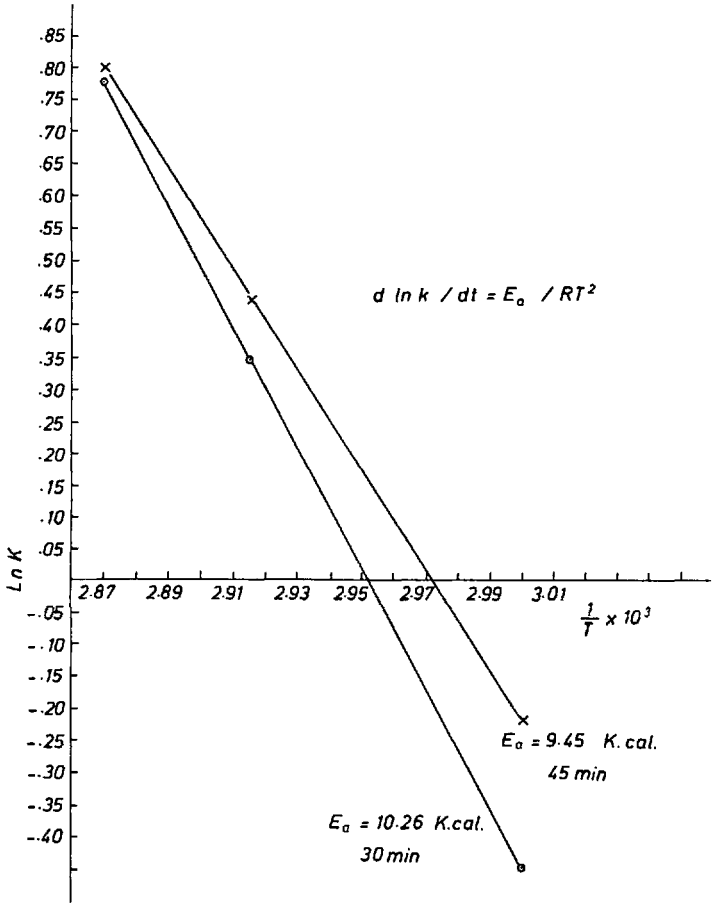

Fig. 1. Arrhenius plot of the natural logarithm of the first order reaction rate constant [K] against the reciprocal of the absolute temperature [1/T].

A plot of natural logarithm of K versus the reciprocal of absolute temperature showed an Arrhenius temperature dependence. The activation energy was calculated from the slope obtained at two periods of exposure. The values differed according to the length of heating and ranged between 9.455 kilocalories and 10.261 Kilo-calories. Lower activation energies were obtained after longer heating periods at the same temperature.

Summary

The water extract of unheated defatted ground soybean (soybean meal) contained haemagglutinin activity 7137 units per g soybean meal and carbohydrate content 6.34 g per 100 g soybean meal. After adjusting the pH-value of the water extract to pH 4.4, and centrifuging it, the supernatant contained most of the carbohydrate content (6.27 g per 100 g soybean meal), while noticeable decrease in the haemagglutinin was found. The DEAE-cellulose chromatography of the acetone precipitate from the water extract of soybean meal gave five protein peaks. The first four peaks showed the presence of haemagglutinin and about 40% of the total haemagglutinin were present in the first peak. The haemagglutinin activity of the water extract of soybean meal was measured at different temperatures for different intervals. The activity was easily affected by the heat treatment and it was destroyed completely after five minutes by heating at 100 °C and the activation energy was 9.45–10.26 kcal/mole.

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Fig. 1. Arrhenius plot of the natural logarithm of the first order reaction rate constant [K] against the reciprocal of the absolute temperature [1/T].