Short Communication

Tadashi HIRATA* and Kazuo FUJITA** : On Amino Acid Constitution of *Poncirus* Seed Protein***

平畑 規*, 藤田一夫** : カラタチの種子タンパク質の構成アミノ酸について

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The properties of the proteins in the seeds were investigated with special reference to their amino acid composition. For detecting individual amino acids, high voltage paper electrophoresis has revealed to be most sensitive and reliable.

Mature seeds of *Poncirus trifoliata* Raf. were used as the materials.

Five g. of the material (eliminated the seed coat) were defatted with ethyl ether. The protein in the residual mass was extracted with 1/30 M. phosphate buffer (pH=7.0), containing NaCl in 10%. The protein in the extract was fractionated by salting out with ammonium sulphate. The flocculent mass of protein formed at each salt concentration was separated by centrifugation of the mixture. Each protein fraction thus obtained was dialyzed for 24 hours against the phosphate buffer, and then concentrated with carbowax. Each protein sample was hydrolyzed at 120° for 3 hours with ten volumes of 1/6 HCl and buffered with formic-acetic acid mixture (pH=1.5). Two μ l. of the sample was subjected to the electrophoresis (7 mA/cm., 100 V/cm.) for 15 min. in the formic-acetic acid medium (pH=1.5), cooled in a *n*-hexane bath. The spots of the constituent amino acids were detected with ninhydrine and determined by comparing with those of the pure samples of amino acids applied on the same sheet. The optical density was measured with a densitometer.

By comparing the density values of the amino acids in each hydrolyzate of the four protein fractions, the following results were obtained.

On the other hand, the mixture of the protein obtained in same way was subjected to the low voltage electrophoresis (0.5 mA/cm., 10 V/cm., sample 0.003 ml.) for 3 hours

Table 1.

Amino acid Sat. of (NH ₄) ₂ SO ₄	Lysine	Ar- ginine, His- tidine	Glycine	Alanine	Iso- leucine, Serine	Leucine	Proline	Phe- nylala- nine, Cystine	Aspar- tic acid
0 ~0.4	1.00	1.63	2.50	3.25	3.62	3.50	3.06	3.44	3.44
0.4~0.5	1.00	1.90	1.38	1.55	2.21	2.17	1.62	2.66	2.62
0.5~0.6	1.00	2.71	2.00	2.00	4.00	4.71	4.00	6.35	5.00
0.6~0.8	1.00	3.00	1.78	3.22	4.56	4.00	4.11	9.78	7.56
Distance of Cataphoresis (cm.)	19.7	18.8	16.0	14.4	12.0	11.7	11.0	10.0	9.2

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in a medium of the phosphate buffer (pH=7.0). The spots of the protein were detected by B.P.B. dyeing, and the optical density was measured with a densitometer. The following Fig. 1 is graphical diagram of the distance of cataphoresis *vs.* the optical densities.

Fig. 1.

The relations between the four hydrolyzated protein fractions in Table 1 and the protein fractions (I, II, III, IV) in Fig. 1 are being investigated.

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