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NOTES

Separation of Amino Acids from Mustard (*Brassica campestris*) Oil Cake

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In the present note the authors attempt to isolate amino acids from mustard oil cake by ligand exchange chromatographic technique.

Oilseed cakes are important source of vegetable proteins. Amino acids are readily formed by hydrolysis of proteins. Different workers have employed microbiological[1,2] and colorimetric methods[3] for determination of amino acids present in oil cakes. Recently, we have successfully employed the well known ligand exchange chromatographic technique[4] for the separation of amino acids from taramira oil cake[5].The present note is an attempt to isolate amino acids from mustard oil cake by same technique.

The residual oil from mustrad oil cake was removed by *n*-hexane. The cake was treated with hot distilled water for 2 hrs. in order to remove the toxic substances present. The proteinous residue left was hydrolysed by refluxing with 6N HCl in sealed tubes at 110°C for 48 hrs. to get hydrolysate containing amino acids along with peptides[6]. The alkali hydrolysis was carried out with 5N NaOH at 110°C for 18 hrs in order to determine tryptophan content which gets destroyed during acid hydrolysis. The amino acids were separated from acid hydrolysed sample by ligand exchange chromatographic technique. The preparation of the metal form of the resin Amberlite IR-120 and the operation of the column was same as discussed previously[5]. The hydrolysed sample after adjusting its pH to 9.5 was applied to the column. The elution was done with 0.1N, 1N, 2N and 3N NH_4OH solutions. The flow rate was set to 5 drops per min. The dicarboxylic amino acids were separated by the method of Darling[7]. Thin layer chromatography in the solvent system (butanol : acetic acid : water : : 4 : 2 : 5) was employed to detect the amino acids present in the different fractions[8]. The various fractions containing same amino acids were mixed accordingly and compared with the standard amino acids by TLC. The amino acids were estimated by the method of Lee and Takahashi[9]. The concentration of amino acids present in the samples was estimated from the Lambert Beer's law plots of standard amino acids. Tryptophan content was determined from alkali hydrolysed sample by the Spies and Chambers method[10]

The crude proteinous residue left after treatment with *n*-hexane and hot water was 63% of the raw material. The residue was hydrolysed with 6N HCl and the hydrolysed sample contained a mixture of amino acids along with the peptides[6]. The amino acid mixture was separated on the ligand exchange columns containing copper (II) form of Amberlite IR–120. This resin contained sulphonic acid groups as active sites. The ligand exchange equilibrium for the process has been discussed earlier[5]. The elution was done with 0.1N, 1N, 2N, and 3N NH_4OH. The mustard oil cake yielded peptide in first 20 ml of the 0.1N NH_4OH. The acidic amino acids, glutamic acid and aspartic acid are eluted in the next 25 ml of 0.1N NH_4OH. The glutamic acid and the aspartic acid are further separated on alumina column. The glutamic acid is eluted with 0.5N acetic acid and the aspartic acid is eluted with 3N KOH and 0.05 N KOH. After the elution of dicarboxylic amino acids, methionine and cystine are eluted with 1N NH_4OH and this is followed by elution of phenylalanine and lysine. The histidine and arginine are eluted with 3N NH_4OH. The elution volume along with the amino acid eluted is given in Table 1. Thin layer chromatography was employed to confirm the amino acids present in the elutant along with the possible standard amino acids. The hRf values of amino acids separated were given in Table 2. The order of elution of amino acids is glutamic acid~aspartic acid> methionine>cystine>phenylalanine>lysine>histidine> arginine. This order is in accordance with the earlier reported order, *i.e.*, a group of acidic amino acid is eluted first of all followed by a group of neutral amino acids and a group of basic amino acids. The weak retention of glutamic acid and aspartic acid can be explained in terms of their low PI values. The amino acids with low PI values are weakly retained by the resin and thereby eluted easily[11]

TABLE 1
LIGAND EXCHANGE CHROMATOGRAPHIC SEPARATION OF AMINO ACIDS FROM HYDROLYSATE OF MUSTARD OIL CAKE

Eluent buffer	Elution volume	Amino acid eluted
0.1N NH_4OH	25 ml	Glutamic acid + Aspartic acid
1N NH_4OH	15 ml	Methionine
	10 ml	Cystine
2N NH_4OH	10 ml	Cystine
	10 ml	Phenylalanine
	5 ml	Lysine
3N NH_4OH	10 ml	Lysine
	10 ml	Histidine
	20 ml	Arginine

TABLE 2
YIELD OF AMINO ACIDS FROM MUSTARD OIL CAKE ON THE BASIS OF PROTEINOUS RESIDUE AND RAW OIL CAKE AND TLC OF AMINO ACIDS ON SILICA GEL-G COATED PLATES

Amino acid	Percentage yield		hRf value of amino acid eluted
	Proteinous residue	Raw oil cake	
Arginine	4.79	3.01	29.0
Aspartic acid	4.91	3.09	30.0
Cystine	3.85	2.42	32.0
Glutamic acid	3.66	2.30	59.0
Histidine	3.92	2.46	41.0
Lysine	6.00	3.78	21.0
Methionine	4.34	2.73	60.0
Phenylalanine	4.11	2.59	58.0
Tryptophan	2.53	1.75	—

The amino acids content of the various fractions was estimated. The mustard oil cake yielded aspartic acid 3.09%, arginine, 3.01%, cystine 2.42%, methionine 2.73%, lysine 3.78%, phenylalanine 2.59% and tryptophan 1.75% (Table 2) on the basis of raw oil cake. The substantial amount of essential amino acids indicated that the mustard oil cake is highly nutritive.

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