Chemical Examination of *Clitoria ternatea* Seeds

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ABSTRACT

Fatty acid composition determined by paper, thin layer and gas liquid chromatography revealed the presence of palmitic, stearic, oleic, linoleic and linolenic acids in the weight ratio of 18.5, 9.5, 51.4, 16.8 and 3.8%, respectively, in *Clitoria ternatea* seed oil. Protein constitutes 38.4% and consists of 18 amino acids. Essential amino acid pattern is (%): lysine (6.55), histidine (2.03), threonine (3.13), phenylalanine + tyrosine (5.5), valine (5.8), methionine + cystine (1.16) and leucine + isoleucine (15.4). The seeds have been found to be rich in calories (500.5 cal/100 g).

INTRODUCTION

Clitoria ternatea is a wild legume of the family Leguminoceae. Earlier workers (1-4) have examined its seed oil content and gave varying compositional data. It has been found to contain 38.4% of proteins and 500.5 cal/100 g of seeds. Because no work has been done on the composition of *C. ternatea* seeds of Madhya Pradesh origin, its calorie composition and nutritional characteristics of its oil and proteins were investigated chromatographically and are reported in this communication.

EXPERIMENTAL

The seeds of *C. ternatea* were extracted with petroleum ether (bp 60-80 C) to yield a greenish-yellow oil in 10.2% yield. Calorie contents, proximate chemical composition of the legume and physicochemical characteristics of the extracted oil were determined by AOAC methods. The results are given in Table I.

Analysis of Oil

The oil was hydrolyzed and mixed acids were isolated and transformed into methyl esters and potassium hydroxamates (5). Fatty acids, their methyl esters and hydroxamates were chromatographed by reversed phase chromatography (5-9) on liquid-paraffin-impregnated Whatman filter paper no. 1 and cellulose thin layer chromatographic (TLC) plates with 85% glacial acetic acid as solvent. Copper acetate rubeanic acid reagent was used for detecting fatty acids and their hydroxamates.

Methyl esters were converted into potassium hydroxamates by spraying with hydroxyl amine and then identified as red spots against yellow background by further spraying the chromatogram with acidified ferric chlroide solution. Unsaturated components were located on the developed chromatogram by exposure to iodine vapors. Methyl esters and hydroxamic acids were resolved by argentation TLC on 12% silver-nitrate-impregnated Silica Gel G plates using petroleum ether/ether (9:1) as solvent. The spots were detected by charring with sulfuric acid. Gas liquid chromatography (GLC) of methyl esters was done in an F&M Model 720 gas chromatograph equipped with a thermal conductivity detector, using DEGS-15% (polyester of diethylene glycol succinate) as the stationary phase on Chromosorb W (40-60 mesh) and nitrogen as carrier gas at a flow rate of 3,600 ml/hr. The temperatures of the column, injection port and detector block were 210, 300 and 300 C, respectively. Five clear peaks of the GLC curve were quantitated by peak area method. The results are given in Table II.

Analysis of Proteins

Proteins were extracted in a 34.0% yield from the defatted seeds with alkaline 10% brine solution and the isolate was hydrolyzed (10). Protein hydrolysate dissolved in 10% isopropanol was chromatographed by various techniques of paper (Whatman no. 1) and cellulose (MN 300) chromatography (11-18) using (a) *n*-butanol/acetic acid/water (4:1:1.6); (b) pyridine/water (4:1); (c) *n*-butanol/pyridine/water (1:1:1) and (d) phenol/water (3:1) as solvents and various specific and multiple spraying reagents (19,20). Amino acids were also resolved on MN Ionex-25 SA-Na cation exchange TLC plates (21) using a 3.2 pH citrate buffer as solvent by ascending run technique. Ninhydrin colors of individual amino acids of the two-dimensionally developed chromatograms were estimated photometrically (22,23) at 570 nm using a Bausch and Lomb Spectronic-20 colorimeter. Amino acids were also quantitated using Perkin-Elmer KLA-38-automatic amino acid analyzer. The results are given in Table III.

RESULTS AND DISCUSSION

Reversed phase paper and cellulose TLC revealed the presence of the usual fatty acids. Argentation TLC of

TABLE I

Characteristics of Legume and Oil

Moisture in seeds (%)	1.75
Oil (%)	10.2
Protein (%)	38.4
Total sugars (%)	44.8
Calorie contents (cal/100 g)	500.5
Ash (%)	3.75
Specific gravity of oil at 30 C	0.8843
Refractive Index at 39 C	1.4590
Saponification value	187.7
Iodine value (Hanus method)	70.4
Acid value	0.25
Unsaponifiable matter (%)	1.8

TABLE II

Fatty Acid Composition of Oil

Serial no.	Component acids	Percentage by weight				
		I	II	III	IV	V
1	Palmitic	18.5	8.8	17.53	[25.8]	6.93
2	Stearic	9.5	3.5	14.24		6.60
3	Oleic	51.4	51.6	35.61	52.3	34.10
4	Linoleic	16.8	15.8	24.1	16.7	35.6
5	Linolenic	3.8	–	8.5	–	–
6	Arachidic	–	1.0	–	–	–
7	Higher saturated acid (C_{20-24})	–	–	–	5.2	10.03

[a]Column I: data of present investigation. Columns II-V: compositional data cited from literature (Refs. 1-4).

TABLE III

Amino Acid Composition of Protein Isolate (Amino Acid Percentage Expressed in g/16 g of Nitrogen)

Serial no.	Amino acid (%)	I[a]	II
1	Lysine	6.55	6.40
2	Histidine	2.03	2.15
3	Threonine	3.13	3.2
4	Phenylalanine	3.30	3.2
5	Valine + glycine	12.88	13.2
6	Methionine	1.06	1.4
7	Leucine and isoleucine	15.51	15.8
8	Serine	6.86	6.7
9	Tyrosine	2.17	2.05
10	Cystine	–	0.11
11	Arginine	7.13	7.16
12	Glutamic acid	24.03	23.9
13	Aspartic acid	12.70	12.5
14	Alanine	4.6	6.8
15	Proline	–	Traces
16	γ-Aminobutyric acid	–	Traces
17	Valine	+	5.8

[a]Data of automatic amino acid analyzer. Column II: compositional data based on spectrophotometric method.

methyl esters and hydroxamic acids gave clear spots of saturated, oleic, linoleic and linolenic acids parallel to their authentic samples resolved alongside, supporting the work of Ansari et al. Observations of reversed phase paper, cellulose and argentation TLC are further supported by five methyl ester peaks of the GLC curve. Quantitation of the GLC curve using the peak area method gave palmitic (18.5%), stearic (9.5%), oleic (51.4%), linoleic (16.8%) and linoleic (3.8%) acids. Differences in compositional data of the oil is probably due to climate and environmental variations of the seed habitats. Two-dimensional paper, cellulose and ion exchange resin TLC showed the presence of 18 amino acids, including eight essential and five semi-essential amino acids. Quantitation by two-dimensional chromatography combined with photometric determination of ninhydrin colors gave results comparable to those obtained by using automatic amino acid analyzer for all amino acids except alanine and methionine. The seed proteins are particularly rich in lysine, phenylalanine + tyrosine, methionine, leucine and isoleucine. Tryptophan appears to be its limiting amino acids.

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