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Proteins from *Benincasa hispida* Fruit Juice: Chemical Characterization Using Total Hydrolysis, Gel Permeation Chromatography and SDS PAG Electrophoresis

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The juice was extracted from the matured fruit *of Benincasa hispida* and proteins isolated there from by precipitation with ethanol. Glutamic acid, aspartic acid, phenylalanine, leucine, threonine, serine, glycine, alanine and valine were the major amino acids present in these proteins. Gel permeation chromatography on Sephadex G-200 of the isolated protein indicates the presence of at least two populations. SDS-PAG electrophoresis study showed the presence of two populations having molecular weights 11,500 Da and 58,900 Da.

Key Words: *Benincasa hispida*, Fruit, Juice, Protein, Amino acid composition, GPC, SDS-PAGE

INTRODUCTION

Benincasa hispida (Thumb) Cogn. locally known as 'Chalkumra' grows commercially in almost every part of India[1]. The extractable juice of the fruit has various uses. It has been used as traditional folk medicine for the treatment of hemorrhagic diseases, epilepsy, prevention of dandruff and other skin diseases[1,2]. It has recently been reported that extracts of the fruit prevent development of experimental ulcers[3]. The fresh juice of *B. hispida* showed anti-inflammatory activity in cotton pellet granuloma and carrageenan-induced edema in rats[4] and was found effective in preventive morphine withdrawal in mice[5]. But active principles responsible for these bioactivities have not been determined. Screening assay from various sources has shown that besides low molecular weight compounds high molecular weight polymeric materials like polysaccharides and proteins have pharmacological activities[6–8]. In one of our studies, one of the major constituents of the juice, the polysaccharide was isolated and its sugar composition was determined[9]. The presence of a linear β-(1→4)-D-galactan, homogalacturonan, arabinan in the alcohol-insoluble residue of *B. hispida* fruit has recently been reported[10]. The aim of the present work is to isolate proteins present in *Benincasa hispida* fruit juice and to investigate its chemical composition, homogeneity and molecular weight.

EXPERIMENTAL

Material and isolation of protein

The fruit was collected from Tentulia village of Birbhum district in West Bengal, India and cut into pieces (*ca.* $0.5 \times 0.3 \times 3$) cm^3. The cut pieces of the mesocarp were homogenized using a household blender and the homogenized tissue zone centrifuged (8000 rpm, 30 min). The centrifugate was then filtered to separate the extractable juice from the solid residue. The concentrated juice was finally diluted with 3 volumes of ethanol. The pellet, isolated by centrifugation (8000 rpm, 30 min) was dissolved in water and lyophilized to yield the polymeric material (P).

Amino acid composition

The freeze-dried protein (P) was hydrolyzed using 6 N HCl containing 0.1% of phenol and 0.05% of β-mercapto ethanol in glass tubes. Tubes were sealed under vacuum and heated at 110°C for 22 h. After the removal of the acid, the content was dissolved in a buffer and analyzed by Pharmacia LKB Alpha plus amino acid analyzer.

Gel permeation chromatography (GPC)

GPC of the protein (P) was carried out through Sephadex G-200 column (2.3×50 cm) as described[11]. Sample (P, 3 mg) was dissolved in 0.3 M sodium acetate buffer (pH 5.0) and passed through the column using the same buffer at *ca.* 20 mL/h. Elution of protein from GPC is expressed as K_{av} $\left[K_{av} = \frac{V_e - V_0}{V_t - V_0}\right]$ where V_t and V_0 are the total volume and void volume of the column determined as the elution volume of glucose and dextran of molecular weight 200,000 Da (Fig. 1). The column was also calibrated using standard dextran.

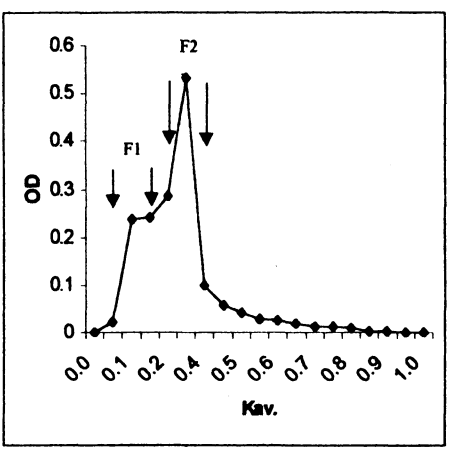

Fig. 1. Size exclusion chromatographic elution diagram of the protein fraction (A) isolated from *Benincasa hispida* fruit juice from a Sephadex G-200 column

SDS-PAG Electrophoresis

Dried sample was dissolved in distilled water and dialyzed against 0.01 M phosphate buffer at pH 7.0 for 48 h. Sample solution containing 5 mg/mL protein was incubated at 98°C for 5 min and then subjected to SDS-PAGE according to the method of Weber and Osborn[12]. Detection was achieved by staining the gels in a solution of Coomassie brilliant blue (1.25 g Coomassie brilliant blue in 454 mL 50% MeOH and 46 mL AcOH) for 2 h followed by destaining electrophoretically using HOAc-MeOH-H_2O (3 : 2 : 35) system. The molecular weights of proteins were determined from a linear curve obtained by plotting log m.w. against mobilities of standard proteins (BSA, ovalbumin, pepsin and lysozyme) as shown in Fig. 2.

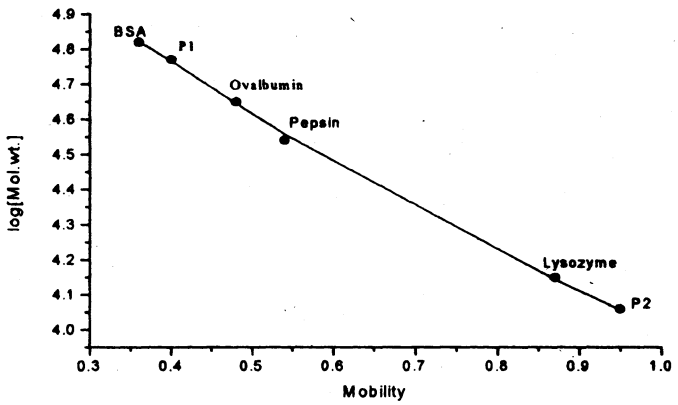

Fig. 2. SDS-PAG electrophoretic mobilities and molecular weight of standard proteins and of fractions obtained from the proteins of *B. hispida* fruit juice

RESULTS AND DISCUSSION

The juice of matured *Benincasa hispida* fruit has numerous pharmacological activities[3–5], but no detailed study has yet been carried out to identify the active principles present therein. In continuation of our effort[9,10] to investigate the chemical constituents present in *Benincasa hispida* fruit, we have extracted juice from the matured fruit using warring blender. A large portion, about 95–97% of the mesocarp, was water but only about 65–67% of this could be extracted. Proteins were isolated from the fresh juice by precipitation with 3 volumes of ethanol. The amino acid composition of the isolated protein has been determined by methods as previously reported[6]. The result as shown in Table-1 reveals the presence of sixteen amino acids, of which nine were essential. Glutamic acid, aspartic acid, phenylalanine, leucine, threonine, serine, glycine, alanine and valine were the major amino acids detected.

The isolated protein has been fractionated by size exclusion chromatography on Sephadex G-200 column (Fig. 1). About 56% of the polymers had K_{av} between 0.24 and 0.36 whereas 30% had between 0.1 and 0.35. Based on calibration with

dextrans, the apparent molecular weight of the first peak (F_1) would be 110,000 Da ± 20,000 while that of the second fraction (F_2) might be estimated to be 70,000 Da ± 30,000.

TABLE-1
AMINO ACID COMPOSITION OF PROTEIN ISOLATED FROM THE JUICE OF *BENINCASA HISPIDA* FRUIT

Amino acids	Weight percentage
Aspartic acid	13
Threonine	7
Serine	8
Glutamic acid	13
Proline	4
Glycine	7
Alanine	7
Valine	7
Methionine	1
Isoleucine	2
Leucine	9
Tyrosine	3
Phenylalanine	11
Histidine	1
Lysine	5
Arginine	3

SDS-PAGE was conducted on the isolated protein for further evaluation of the molecular weights of the protein fractions. The protein isolate showed two bands (P_1 and P_2). Mobilities of these protein bands were determined (Fig. 2) and compared with standard proteins (BSA, ovalbumin, pepsin and lysozyme). Molecular weights of the components on the basis of their mobilities (Table-2) were 58,900 Da (P_1) and 11,500 Da (P_2).

TABLE-2
MOLECULAR WEIGHT DETERMINATION BY SDS-PAGE

Proteins	Mobility	m.w. (Da), Literature values	m.w. (Da), Experimental results
BSA	0.36	66,000	
Ovalbumin	0.48	45,000	
Pepsin	0.54	34,700	
Lysozyme	0.87	14,300	
P_1	0.40		58,900
P_2	0.95		11,500

The two components or bands thus obtained from SDS-PAGE may be due to either two different proteins or different subunits of the same protein. The apparent molecular weights of proteins as calculated from SDS-PAGE are significantly different from those obtained by size exclusion chromatography. However, it should be noted that proteins containing charged amino acid (aspartic acid, glutamic acid, lysine, histidine), due to intramolecular electrostatic interactions by charge effects, may have a different hydrodynamic volume than dextrans and, therefore, elute at a different rate than expected on the basis of their molecular weight. Future studies will throw light on whether these proteins are responsible for the biological activities of the juice.

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