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EVALUATION OF BIOCHEMICAL COMPOSITION AND PHYSICOCHEMICAL PARAMETERS OF OIL FROM SEEDS OF DESI CHICKPEA VARIETIES CULTIVATED IN ARID ZONE OF PAKISTAN

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Abstract

Seeds of three approved desi chickpea (*Cicer arietinum* L) varieties viz., Punjab 2000, Bittal 98 and CM 72 grown in arid zones of Pakistan were analysed for their biochemical and physicochemical compositions. The moisture contents ranged from 6.30±0.40-7.60±0.30 g/100g, dry matter from 92.30±0.69-92.70±0.41 g/100g, ash from 3.10±0.01-3.22±0.02 g/100g, crude protein from 22.19±0.11-22.50±0.43 g/100g, crude fat from 2.05±0.04-2.10±0.04 g/100g, total carbohydrates from 64.90±0.16-66.51±0.11 g/100g and calorific value from 368-373 Kcal/100g. The content of total nitrogen ranged from 3.55±0.11-3.60±0.43 g/100g. SDS-polyacrylamide gel electrophoresis studies showed 5 bands of different proteins in each variety. All of the 3 varieties were found to be almost similar on the basis of total sugars, free amino acids and ascorbic acid contents. The crude fibre content ranged from 13.2±0.11-14.10±0.54 g/100g. The oil from the seeds of each chickpea variety was also analysed for specific gravity (0.9339-0.9346), acid value (2.40±0.87-2.50±0.67 mg KOH), iodine value (112.56±0.371-13.87±0.29), saponification value (178.90±0.19-180.64±0.12 mg KOH) and unsaponifiable matter (3.42±0.97-3.47±1.02 g/100g). These three varieties showed significant variation in the moisture, ash contents, total carbohydrates, iodine value and saponification value. The research results about the biochemical characteristics of desi chickpea varieties are expected to provide guidelines for the researchers confronted with the need to use such typical food seeds in Pakistan as well as in the rest of the world.

Introduction

Pakistan is basically an agricultural country and almost 70 % of its economy, directly or indirectly, is dependent on the agricultural sector. The government is paying due attention to this sector and plant breeders are introducing those varieties which are highly productive and have improved nutritional composition. Legume crops, "the meat of poor", are cultivated on a large area of Pakistan. Legumes, being rich sources of protein, calories, certain minerals and vitamins play an important role in human nutrition (Deshpande, 1992). Food legumes are crops of the family Leguminosae also called Fabacae. World widely, these are mainly grown on large area for their edible seeds and thus are also named grain legumes. Amjad *et al.,* (2006) reported that legumes are helpful in enhancing the protein content and improving the nutritional status of the cereal-based diets. Amjad *et al.,* (2003) investigated that cereal proteins are deficient in certain essential amino acids, particularly lysine. On the other hand, the work of Farzana & Khalil (1999) has shown that cereal proteins contain an adequate amount of lysine but deficient in methionine, cystine and cysteine.

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Chickpea (*Cicer arietimum* L.) holds a prominent position among legume crops and is the most abundant crop grown after wheat and rice for food purpose in Pakistan. Cowan *et al.,* (1967) reported that chickpea is a good source of iron and its availability is highest as compared to other food legumes. Chickpea being one of the major pulse crops in Pakistan, it is usually grown on the sandy characteristic soils of the country where no other crops of the rabi season can grow (Khattak *et al*., 2007). Chickpea growers of the irrigated as well as arid areas require genotypes with a relatively high yield so that they may compensate their needs. Farmers of the irrigated areas may be able to get not only benefit from restoring nitrogen deficiency in the soil, but also they can get cash return to fulfill their daily requirements after crop harvesting (Khattak *et al*., 2007).

Qureshi *et al*., (2004) carried the studies to find out the variability for economically important traits in chickpea (*Cicer arietinum* L.) Different chickpea varieties showed genetic variability for plant height, number of primary branches, number of secondary branches, number of pods per plant and total biological yield. These studies are helpful to develop high yielding varieties. Since the biochemical composition of crops varies with crop cultivars, soil and climatic conditions of the area, it is imperative to study the biochemical composition of different varieties of desi chickpea. Recently, Haq *et al.*, (2007) have performed studies on the physical characteristics, mineral composition and distribution patterns of various amino acids and fatty acid profile of seeds of some desi chickpea cultivars grown in Punjab, Pakistan. But the proximate composition like sugars, proteins, carbohydrates etc., of these varieties have not been investigated. There is, still a need to evaluate these characteristics so that, like other leguminous crops, chickpea may be helpful in solving the protein-calorie malnutrition problem all over the world. Therefore, in the present studies the various biochemical parameters *i.e.,* moisture, ash, sugars, amino acids, total nitrogen, total protein, oil, ascorbic acid and crude fibre contents of various desi chickpea varieties have been analysed.

Materials and Methods

Samples: Seeds of three improved varieties of desi chickpea (*Cicer arietinum* L.) viz., CM 72, Bittal 98 and Punjab 2000, recommended for cultivation in arid zone and irrigated regions of Pakistan, were collected from Pulse Section, Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan. The seeds were cleaned, freed from dust and other foreign materials and stored at room temperature in the air tight plastic jars for analysis.

Moisture contents: The moisture and dry matter contents of the samples were determined by following the standard method of AOAC (Anon., 1990).

Ash contents: The ash contents were determined by the method as described by Pearson (1980).

Nitrogen and proteins: The contents of total nitrogen in each variety were estimated by using the Kjeldahl procedure (Anon., 1990). The percentage of crude proteins was calculated by multiplying the percent nitrogen by 6.25. The water soluble and salt soluble (0.5 M $(NH_4)_2SO_4$) protein contents were determined by Biuret method (Plummer, 1979).

Polyacrylamide gel electrophoresis (PAGE) of proteins: For electrophoresis, seed proteins were extracted following the method reported by Ng & Bushuk, (1987) and analysed by SDS-polyacrylamide gel by the method of Laemlli (1970) with 10 % monomer concentration. The MW markers employed were: β –lactoglobulin (18400 Da), trypsinogen (24000 Da), pepsin (37400 Da), egg albumin (45000 Da) and bovine albumin (66000 Da).

Sugars and free amino acids: One gram of finely ground seeds of each of three desi chickpea varieties was soaked separately in 75% ethanol (25 ml). After 24 hours, the samples were ground and filtered. The filtrates were collected into three different 100 ml measuring flasks. The residue was washed with a few ml of 75% ethanol in each case and washings were also collected into respective flasks. The volume was made up to the mark in each case by adding 75% ethanol. These extracts were used for the estimation of sugars and free amino acids.

Total sugars and reducing sugars were estimated by the methods of Travelyan & Harrison (1952) and Hulme & Narain (1931), respectively. Non-reducing sugars were calculated by the difference between total sugars and reducing sugars. Qualitative analysis of sugars was carried by paper chromatography. The chromatograms were developed using solvent system, ethyl acetate-pyridine-water (60:30:20) and aniline phthalate as locating agent.

Total carbohydrates and the calorific values were calculated by the method used by Onyeike *et al.*, (1995). The contents of free amino acids were measured following the method of Hamilton & Slyke (1943).

Ascorbic acid and crude fibre: Ascorbic acid content was determined using the method as described by Rankin & Hildreth (1976) and crude fiber contents were determined by AACC (Anon., 1983) approved method.

Content and physicochemical characteristics of oil: Extraction of oil was done by soxlet apparatus using n-hexane as solvent (Kanwar & Pushpamma, 1980). Density of oil was determined by using pycnometer and its specific gravity was calculated by following formula:

$$\text{Specific gravity} = \frac{\text{Density of oil}}{\text{Density of water}}$$

The acid value, iodine value and saponification value were determined by following the method as described in British Pharmacopeia (1973) while the determination of unsaponifiable matter was carried out by using the method as described in US Pharmacopeia (1985).

Statistical analysis: The results were presented as mean values ± standard deviation of the three replicates. Data were statistically analyzed using Minitab Software, version 13.3. The significant difference between means was calculated by one way ANOVA ($p \leq 0.05$).

Results and Discussion

Biochemical composition: The biochemical composition of the seeds of three desi chickpea varieties is presented in Table 1. The moisture contents in these varieties ranged from 6.30 to 7.60 g/100g. They were found to be high in CM 72 (7.60 g/100g) followed by Bittal 98 (6.70 g/100g) and Punjab 2000 (6.30 g/100g). A significant difference was observed among the varieties ($p<0.05$). The results were close to those reported earlier (Amjad *et al.,* 2006; Benu & Shrivastava, 2006) but were not in good agreement with those reported by other workers (Lal, 1950;Sinan & Aysegul, 1977). No significant variation was observed in the calculated values of dry matter (92.30±0.69-92.70±0.41g/100g). The ash contents ranged from 3.10±0.01 to 3.22±0.02 g/100g, showing significant difference among the varieties ($p<0.05$). These results were comparable to those investigated by earlier workers (Lal *et al.,* 1964; Khan *et al.,* 1995; Amjad *et al.,* 2006; Benu & Shrivastava, 2006; Amir *et al*., 2007).

The nitrogen and protein contents ranged from 3.55±0.11 to 3.60±0.43 g/100g and 22.19±0.11 to 22.50±0.43 g/100g respectively. No significant difference appeared among the varieties. The nitrogen contents were not in agreement with those reported earlier (Lal, 1950; Azimou, 1970). The observed values for protein contents were slightly less than those reported in literature (Khan *et al.,* 1995; Amjad *et al.,* 2006 & Amir *et al*., 2007) but these were found to be relatively high than those reported by other workers (Sinan & Ayesgul, 1977; Gloria, 1980; Kamal, 1991; Benu & Shrivastava, 2006). The higher protein contents indicate that chickpeas are truly called "meat of the poor". The water soluble, salt soluble and total salt soluble protein fractions from the chickpea seed flour were also obtained and the results have been summarized in Table 1. No significant variation among the varieties was observed for protein contents in case of each fraction. Higher protein contents were found in salt soluble fraction of each variety without any significant variation.

Crude fat ranged from 2.05±0.04 g/100g (CM 72) to 2.10±0.04 g/100g (Punjab 2000) with no significant difference among the varieties. All of the three chickpea varieties were investigated as the poor source of crude fat. The results were found to be relatively low as compared to those already reported in literature (Joshi & Ram, 1972; Krivelevich, 1982; Dodak *et al.,* 1993; Bhandari *et al.,* 2003; Amir *et al.,* 2007) but fall in the wide range as indicated by Sinan & Ayesgul (1977).

Total carbohydrates determined by difference were found to be relatively high in Punjab 2000 (66.51±0.11g/100g) followed by Bittal 98 (66.05±0.20 g/100g) and low in CM 72 (64.90±0.16 g/100g). Statistically significant difference was observed among the three varieties ($p<0.05$). The sugar contents *i.e.,* total sugars (5.00±0.70-5.01±0.33 g/100g), reducing sugars (1.70±.66-1.70±.33 g/100g) and non-reducing sugars (3.30±0.77-3.30±0.33 g/100g) were found to be insignificantly different among the varieties. Non-reducing sugars contents were found to be higher than those of reducing sugars in each case. Table 2 presents the qualitative analysis of sugars in the seeds of the three chickpea varieties. Three sugars viz., glucose, fructose and xylose appeared in each case.

The calorific value was calculated by multiplying the mean values of crude proteins, crude fat and total carbohydrates by Atwater factors of 4, 9 and 4 respectively, taking the sum of the products and expressing the results in Kilocalories/ 100 g of samples. The calorific value (Kcal/100g) was highest in Punjab 2000 (373) followed by Bittal 98 (371) but lowest in CM 72 (368). The calculated carbohydrates and the calorific values were found to be relatively high as compared to those reported earlier for desi chickpea (Khan *et al.,* 1995). This variation may be attributed to climatic and varietal differences.

Table 1. Biochemical composition of seeds of different chickpea varieties

Components (g/100g)	CM 72	Bittal 98	Punjab 2000
Moisture	7.60 ± 0.30	6.70 ± 0.72	6.30 ± 0.40
Dry matter	92.40 ± 0.35	92.30 ± 0.69	92.70 ± 0.41
Ash	3.10 ± 0.01	3.22 ± 0.02	3.15 ± 0.01
Total nitrogen	3.60 ± 0.43	3.56 ± 0.23	3.55 ± 0.11
Crude protein (Nitrogen×6.25)	22.50 ± 0.43	22.25 ± 0.23	22.19 ± 0.11
Water soluble proteins	9.00 ± 0.44	9.00 ±0.33	9.00 ± 0.77
Salt soluble proteins	10.00 ± 0.77	10.00 ± 0.44	10.00 ± 0.65
Total salt soluble proteins	22.00 ± 0.55	22.00 ± 0.33	22.00 ± 0.23
Crude Fat	2.05 ± 0.04	2.07 ± 0.03	2.10 ± 0.04
Total carbohydrates	64.90 ± 0.16	66.05 ± 0.20	66.51 ± 0.11
Sugars			
Total sugars	5.00 ± 0.70	5.01 ± 0.33	5.00 ± 0.23
Reducing sugars	1.70 ± 0.33	1.70 ± 0.66	1.70 ± 0.45
Non-reducing sugars	3.30 ± 0.77	3.30 ± 0.33	3.30 ± 0.55
Calorific value (kca/100g sample)	368	371	373

Values are the means ± SD of three determinations.

Table 2. Qualitative analysis of sugars in the seeds of different chickpea varieties.

Standard sugar	Rf Values of standards	Varieties		
		CM 72	Bittal 98	Punjab 2000
Fructose	0.59	+	+	+
Galactose	0.36	–	–	–
Glucose	0.56	+	+	+
Xylose	0.65	+	+	+

+ = Present, – = Absent

Solvent system; ethyl acetate-pyridine-water (60:30:20)

Locating reagent; phthalic anhydride-aniline solution in methanol-ethanol mixture

The results of analysis of free amino acids, crude fibre and ascorbic acid are given in Table 3. No significant difference was found in the contents of free amino acids, crude fibre and ascorbic acid among the three varieties. The observed ascorbic acid contents were found to be close to the values reported by Sood & Malhotra (2001). The crude fibre contents were found to be high than those reported earlier (Lal *et al.*, 1964; Khan *et al.*, 1995). These varieties are high in dietary fibre and hence a healthy source of carbohydrates for persons with insulin sensitivity or diabetes. The results obtained in the present study are significant to the extent that the presence of high crude fibre in the three chickpea varieties may enhance their digestibility and also aid the peristaltic movement of the intestinal tract (Davidson & Passmore, 1972).

Polyacrylamide gel electrophoresis (PAGE) of proteins: The pattern of proteins in the seeds of three chickpea varieties analysed by SDS-polyacrylamide gel electrophoresis are shown in Fig. 1. In total, 5 bands were observed in the seeds of each variety. Protein sample of each variety showed, 62,000, 38,000 and 20,000 Da bands, which correspond to the appropriate molecular weights of standard proteins. The amount of proteins having molecular weight, 20,000 Da was found to be higher in Bittal 98 than those of CM 72 and Punjab 2000. No other specific variation in SDS-PAGE electrophoretogram of the total proteins in three chickpea varieties was detected. It is clear from the results obtained that the seeds of all three varieties contain proteins of the same molecular weight. Also the concentration of all other bands of proteins is almost similar in all of the three varieties investigated. It is, therefore, not possible to distinguish the varieties by this technique as also reported earlier by Singh *et al.*, (1981).

Table 3. Free amino acids, crude fibre and ascorbic acid contents of seeds of different chickpea varieties.

Components	CM 72	Bittal 98	Punjab 2000
Free amino acids (g/100g)	0.40 ± 0.04	0.40 ± 0.01	0. 40 ± 0.02
Crude fibre (g/100g)	13.2 ± 0.11	13.7 ± 0.97	14.10 ± 0.54
Ascorbic acid (mg/100g)	2.00 ± 0.02	2.00 ± 0.01	2.00 ± 0.02

Values are the means ± SD of three determinations.

Table 4. Physicochemical characteristics of oil from the seeds of different chickpea varieties.

Parameter	CM 72	Bittal 98	Punjab 2000
Specific gravity	0.9346	0.9339	0.9343
Acid value (mg KOH)	2.40 ± 0.87	2.50 ± 0.67	2.42 ± 0.53
Iodine value	113.55 ± 0.32	112.56 ± 0.37	113.87 ± 0.29
Saponification Value (mg KOH)	180.64 ± 0.12	179.21 ± 0.07	178.90 ± 0.19
Unsaponifiable matter (g/100g)	3.47 ± 1.02	3.45 ± 1.02	3.42 ± 0.97

Values are the means ± SD of three determinations

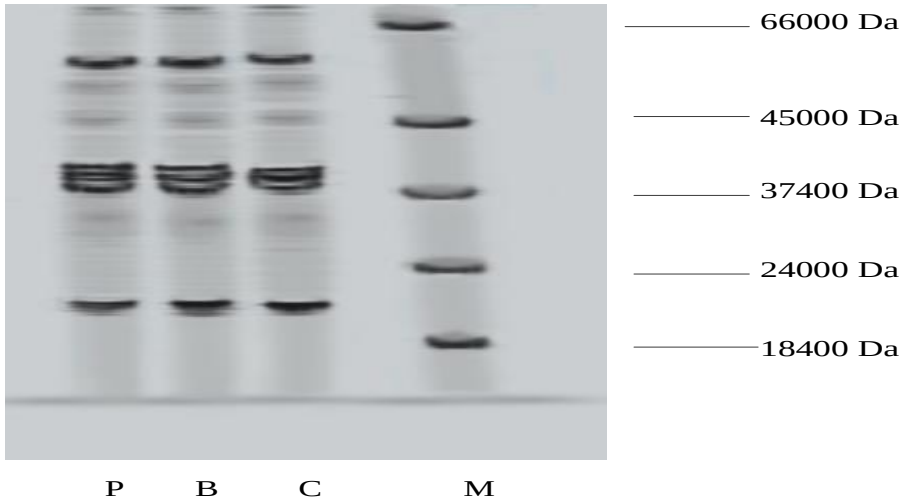

Fig. 1. Electrophoretic pattern of the proteins in the seeds of different chickpea varieties; P: Punjab 2000, B: Bittal 98, C: CM 72, M: Marker.

Physicochemical characteristics of oil: The results of the physicochemical analysis of the oil have been summarized in Table 4. The results revealed that specific gravity and acid values ranged from 0.9339 to 0.9346, and 2.40±0.53 to 2.50±0.67 mg KOH respectively. The iodine values ranged from 112.56±037 (Bittal 98) to 113.87±0.29 (Punjab 2000) and saponification values from 178.90±0.19 mg KOH (Punjab 2000) to 180.64±0.12mg KOH (CM 72). These two parameters were found significantly different among the three varieties (p<0.05). Unsaponifiable matter ranged from 3.42±0.97 to 3.47±1.02 g/100g. Among the three varieties, Bittal 98 showed higher acid value but was low in iodine value and specific gravity. On the other hand CM 72 was found to be high in specific gravity, saponification value and unsaponifiable matter contents while Punjab 2000 was found to be high in iodine value. These results are comparable to those reported earlier by Bhandari *et al.*, (1950).

In the present study, the results show that the varietal differences are significant regarding moisture content, ash content, total carbohydrates, iodine value and saponification value. The study may need some further statistical analysis, however, the present work about the biochemical characteristics of desi chickpea varieties is expected to provide guidelines for the researchers confronted with the need to use such typical food seeds in Pakistan as well as in the rest of the world.

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