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Original Article

Characterization and Biochemical Studies of the Oils Extracted from Four Cultivars of *Vigna mungo,* Grown in Pakistan

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ABSTRACT

Objective: *Vigna mungo* (mash) is a widely consumed legume in Pakistan and also used globally. This study was carried out for detailed characterization of oils from mash seeds from four indigenously cultivated varieties, as very little information is available on the oil composition of mash seeds and inter-varietal variation in oil composition. **Materials and Methods**: Morphological study was done for each cultivar prior to extraction and characterization of mash seed oil by conventional methods. Seed volume, seed density, hydration capacity, hydration index, swelling capacity, swelling index was determined for mash seeds of each of four cultivars. Oil extraction was done by soxhlet apparatus and characterization of oil for different ingredients was done using GC-MS and HPLC. **Results:** The oil content was relatively low (1–2%). The investigated physiochemical parameters included refractive indices (RI) at 40 C (1.6–1.9), relative density (1), iodine value (IV) (114.3 –117.1), saponification value (SV) (156.0–167.4 mg KOH/g) and unsaponifiable matter (UM) (14.3-15.3%). Tocopherol contents were also determined and each cultivar was found to contain appreciable amounts of these constituents (Table 4).The concentration of **ã-tocopherols** were found to be greater than the δ-and á-tocopherol which is quite different than mungbean.. Bulk chemical properties such as acid value (AV), saponification value (SV), iodine value (IV), PV, and p-anisidine value (pAV) give structural, stability, and quality information about oils and fats. The UM ranged from 14.3 to 15.3 (Table 2) and it was revealed that cultivars differed significantly with respect to UM. These values are in consistent with the UM pattern observed for low oil-bearing seeds. **Conclusion:** The findings of the study showed that mash [*Vigna mungo*(L.) Happer], is a potentially valuable legume crop with considerable nutritional quality oil among all the cultivars. Fatty acid profile of mash seed oil showed the presence of **linoleic, and oleic acids** as prominent fatty acids. The bioactive components need to be tested using in-vitro assays for a better understanding of the mechanisms which are targeted by these products to inhibit carcinogenesis.

Introduction

Dry legumes constitute one of the richest and least expensive sources of proteins in the human diet in many parts of the world. Black gram or mash [*Vigna mungo* (L.) Hepper] is an important leguminous crop. It belongs to the family *Leguminoseae* which now a days named as *Fabaceae* but the former one is also valid at present, and genus *Vigna.* Black gram or mash [*Vigna mungo*

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(L.) Hepper] is an important summer pulse crop of many South Asian countries including Pakistan, India, Nepal, Bangladesh, Thailand, Philippines and Korea [1, 2], [3,4].

Chemical analysis of mash seeds indicates that it contains 10% water, 22–24% protein, 2.1% oil, 1–2% fats, 50–60% carbohydrates, and a fair amount of vitamin A and B[5]. Black gram like other legumes contains raffinose family oligosaccharides, among the soluble sugars, raffinose oligosaccharide constitute for 31–76% of them [6]. Mash bean oil consists of tocopherols and tocotrienols[7]. Both these constituents have their own nutritional and medicinal importance. The main function of á-tocopherols is that of being a radical chain-breaking antioxidant in membranes and lipoproteins as well as in foods[8]. Due to its antioxidant potential and various functions at the molecular level, it is believed to reduce the risk of cardiovascular diseases and of certain types of cancer [9]. ã-tocopherols have been reported to be more potent than á-tocopherols in decreasing platelet aggregation, LDL oxidation, and delaying intra-arterial thrombus formation [10]. The antioxidant properties of tocotrienols have been reported to be significantly higher than those of tocopherols and may have biologically important properties such as inhibition of cholesterol biosynthesis [11] and are discussed in the context of reducing the risk of breast cancer [12]. Hence concurrent administration of various tocopherols and tocotrienol may result in increased antioxidant, antitumor and potential [13]. In this study four different cultivars were selected and their oils were extracted and analyzed. The oil content was found 1-2%. The fatty acids and tocopherols were also been isolated and their intervarital comparison was done. Three types of tocopherols were determined in each variety i.e. á-tocopherols, ã-tocopherols and δ-tocopherols.

Material and Methods

Seed Samples four different genotypes of black gram (*vigna mungo*) were collected from The Ayub Agricultural Research Centre. The four genotypes were: Mash- 95019, ES-1, Mash -6202-7, Mash-6036-7, and Mash-6036-24. Three random samples of 100 seeds from each genotype per replication were weighed and the values converted to grams per seed. Seed volume was determined by transferring 100 seeds into a 100 ml measuring cylinder, and 50 ml of distilled water were added. The gain in volume divided by 100 was taken as the seed volume. Seed density was calculated as seed weight divided by seed volume. Hydration capacity was recorded as gain in weight after over night soaking in distilled water. Hydration capacity was recorded as gain in weight after overnight soaking in distilled water. Hydration index was calculated as hydration capacity divided by original seed weight. The swelling capacity was determined as gain in volume after overnight soaking in water, and swelling index was calculated as swelling capacity divided by original seed volume. Moisture, lipid, ash, and crude-fiber contents were determined following the standard methods of the Association of Official Analytical Chemists [14]. The organic nitrogen content was quantified by the Kjeldahl method, and an estimate of the crude protein content was calculated by multiplication of the organic nitrogen content by a factor of 6.25. The two different samples were analyzed in triplicate. Total carbohydrate content was calculated from the difference, applying the formula: Total Carbohydrate = 100% - (% protein + % lipid + %ash+ %fiber). The seeds of all selected genotypes were milled gently in a blender (Braun Multimix System 200, with Multimix deluxe grinder, MXK4 Germany), and oil was extracted by Soxhlet apparatus using petroleum ether 40–60 ^{0}C and determined following a previously described method. The oil content was determined as a percentage of the extracted oil to the sample weight (w/w). The samples were analyzed in triplicate, and then mean and standard deviation were calculated. The extracted oils were stored in a cold room (4 ^{0}C) in a dark glass bottle under nitrogen blanket for further analysis. Black gram seeds of four genotypes were dried in oven at 150 ^{0}C to constant weight. Dried seeds were ground to flour, sieved and were taken in the butt tube of soxhlet extraction apparatus to extract the oil with petroleum ether. It was then distilled off completely, the oil weighed and the % oil was calculated. Physiochemical properties of black gram seed oil was studied and the finding showed the difference between the nutritional and anti nutritional contents with the genetics of plant, as the characterization of different oil contents were compared among the different cultivars of black gram. The aim of our research work was to establish the correlation between the contents (nutritional and antinutritive) and genetics i.e. that different varieties have different values of contents. Using high-throughput technologies, relationships between genotype and bioactive ingredients of crops have been investigated. M.B. Mabaleha and S.O. Yeboah characterized and did compositional study of oil of different cultivars of Phaseolus vulgaris, it was concluded that primary FA components of legumes generally are palmitic (16:0), oleic (18:1n-9), linoleic (18:2n-6), and linolenic (18:3n-3) acids. However it is also relevant to mention that external/environmental factors are also important. Samples of oil were methylated. Briefly, oil was hydrolyzed using methanolic potassium hydroxide and resulting fatty acids were converted to their methyl esters using boron trifluoride as a catalyst. The methyl esters were extracted into hexane and analyzed by gas chromatography (GC) using a Perkin Elmer Autosampler XL GC (Perkin Elmer Instruments, Norwalk, CN) with a flame ionization detector (FID). Fatty acids were quantified according to AOCS Official

Method Ce 1-62 where each fatty acid was expressed by using the peak area percent as a ratio to the total area of all methyl esters present [15].

Black gram seeds were analyzed for the fatty acids and are reported in the following table showing the types of fatty acids in each genotype. The oxidative stability is related to the induction period. Induction period is time period at which there is less deterioration of lipids. All four different oil extracts were compared with reference to their induction period and in result their oxidative stability. Triacylglycerol is the basic unit of lipid. The TAG was determined by HPLC. The black gram oil was dissolved in acetone, and 10 ìL was injected in an HPLC system (LC 30; PerkinElmer) equipped with a refractive index detector and an RP18 column. The mobile phase was a mixture of acetone and acetonitrile (50:50, vol./vol.) [16]. The sterol fraction of black gram oil was determined by GC after pretreatment (20). The sterols were then analyzed in a Hewlett-Packard 5890 Series II chromatograph (Palo Alto, CA) with a split/split less injector and an FID. A Hewlett-Packard 3396 Series II integrator was used for data collection. The column was an HP5 (5% biphenyl, 95% dimethylpolysiloxane), 50 mm and 0.32 mm, and the thickness of the film was 0.17 ìm. The injector temperature was 200ºC, the detector temperature 250ºC, and the column temperature was held at 275ºC. The carrier gas was N_2 at 2 mL/min. The PV, expressed as meq peroxide/kg sample, was determined by the iodine titration method. Extracted oil samples (2 g) were weighed into test tubes. The oxidation of the potassium iodide, in acetic acid medium, by the active oxygen of the fat was followed by titration of the free iodine with sodium thiosulfate, using starch as indicator. By calculating the peroxide value the peroxides in particular oil is measured. Samples of oil weighed 100 grams and were diluted with hexane according to the method of Hashim et al.[17]. The samples were accurately weighed and density was used to factor the final volume for content calculation. Tocopherols were analyzed using AOCS approved method. The tocopherols were identified by comparison with standards purchased from Sigma (Sigma Chemical, St. Louis, MO). Standards of á, â, ã and δ tocopherol were diluted with hexane. Their concentration was determined by the absorbance maximums of the solutions using UV spectroscopy according to Beer's Law. Extinction coefficients were taken from the Merck Index. Calculations of the unknown were done by comparison of peak areas and the calculated concentrations of the standard solutions.Black gram seed oil contained á, ã and δ tocopherols which are listed below:

The contents of dry matter and total nitrogen were determined according to procedures described by [18]. The content of amino acids (except for tryptophan) in de-hulled black gram seeds of each genotype was determined using Amino Acid Analyzer (L-8900 Hitachi-hitech, Japan) under the experimental conditions recommended for protein hydrolysates. Samples containing 5.0 mg of protein were acid hydrolyzed with 1.0 mL of 6 N HCl in vacuum-sealed hydrolysis vials at 110 ^{0}C for 22 h. The ninhydrine was added to the HCl as an internal standard. Hydrolysates were suitable for analysis of all amino acids. The tubes were cooled after hydrolysis, opened, and placed in a desiccators containing NaOH pellets under vacuum until dry (5–6 days). The residue was then dissolved in a suitable volume of a sample dilution Na–S buffer, pH 2.2 (Beckman Instr.), filtered through a Millipore membrane (0.22-lm pore size) and analyzed for amino acids by ion-exchange chromatography in a Beckman (model 7300) instrument, equipped with an automatic integrator. Nitrogen in amino acids was determined by multiplying the concentration of individual amino acids by corresponding factors calculated from the percentage N of each amino acid [19]. The ammonia content was included in the calculation of protein nitrogen retrieval, as it comes from the degradation of some amino acids during acid hydrolysis [20, 21]. The ammonia nitrogen content was calculated by multiplying the ammonia content by 0.824 (N = 82.4% NH3).

Statistical Analysis

The analyses were performed with three replicates. The mean values and standard deviation (mean ± SD) were calculated and tested using the Student's t-test (P\0.05). Statistical analysis of variance (ANOVA) was performed on all values using the statistical program Statistical Graphics System version 4.0.

Results and Discussion

The four cultivars of mash were taken from Ayub agricultural research Institute, Faisalabad, Pakistan. The seeds were then grown at agricultural college university, Sargodha for growing each genotype under controlled environment as under same set of soil, climate, light and same irrigation water was provided to each genotype. The crop was harvested at the end of June. The seeds of each genotype were removed from the pods by conventional means and were kept separately in air tight transparent polythene bags. Physical characteristics of seeds i.e. seed weight, seed volume seed density, hydration capacity; hydration index, swelling capacity and swelling index were studied and each genotype has shown considerable difference in all above characteristics. All that characteristics per 100 seeds with respect to mash cultivars are reported in Table 1.

Proximate composition of mash seeds for each genotype was determined which also showed the considerable difference as studied previously that variety had a significant effect on protein, starch and ash content reported by Wang and Daun (2004). The moisture, ash, fiber, dietary fiber (soluble and

insoluble), protein, carbohydrate content were determined and are reported in Table 2.

The oil was extracted from each cultivar of mash seeds from Soxhlet extraction, the more efficient of the two extraction methods, were very low and thus upheld the view that, black gram are generally not oil-bearing seeds. The oil yields for the four cultivars ranged from 1.9 to 2.8 the results revealed that the cultivars differed significantly with each other as far as total oil contents are concerned.

Bulk chemical properties such as acid value (AV), saponification value (SV), iodine value (IV), PV, and p-anisidine value (pAV) give structural, stability, and quality information about oils and fats. These values are reported in Table 2. Moisture content also showed considerable difference among the mash cultivars and ranged from 10.21 to 12.21. The relative densities of each cultivar of mash seed oil was found to be same which showed that although the other parameters show variations among the cultivars but the densities of oil remains same. Similarly refractive index of oil content for mash cultivars were more or less same as M1 (1.9) showed slight variation from rest of three cultivars (1.6).

The UM ranged from 14.3 to 15.3 (Table 2) and it was revealed that cultivars differed significantly with respect to UM. These values are in consistent with the UM pattern observed for low oil-bearing seeds [22].

Table 1: Morphological characteristics of mash seeds

Properties	95019	6202-7	6036-7	6036-24	ES-1
Seed volume(cm^3/100seeds)	0.120	0.095	0.095	0.080	0.040
Seed density (g/ml)	0.458	0.547	0.589	0.663	1.40
Hydration capacity (g/seed)	0.057	0.041	0.029	0.035	0.013
Hydration index	1.0364	0.788	0.518	0.661	0.232
Swelling capacity (ml/seed)	0.12	0.095	0.095	0.08	0.04
Swelling index	2.182	1.826	1.696	1.509	0.714

Table 2: Physiochemical properties of cultivars of black gram seed oil

Properties	M1	M2	M3	M4
Oil	1.9	2.4	2.8	2.5
Moisture	12.21	11.91	10.21	11.34
Relative density	1	1	1	1
Refractive index	1.9	1.6	1.6	1.6
Unsaponifiable matter	14.3	14.7	15.3	14.9
Saponifiable value	167.4	167.1	156.0	161.1
I.V	117.1	114.3	115.4	115.7

The FA composition of the black gram cultivars investigated (Table 4), despite differences among cultivars, followed the general pattern for legumes [23]. with linoleic and oleic acids as the dominant FA (Table 3).The seed oils from the black gram cultivars investigated contained significant amounts of saturated FA, (16:0), a common feature of legume seed oils [24], which was confirmed by the FA profile of the mungbean cultivars investigated previously and now this characteristic is also confirmed by the FA profile of black gram seeds. Linoleic and linolenic acids are the most important essential FA required for growth, physiological functions and maintenance [25] Thus, consumption of the black gram cultivars, in addition to providing nutrients such as proteins, carbohydrates, and minerals, must also impart some of the widely acclaimed health benefits of these FA to the indigenous population of Pakistan. The FA composition of the oils largely corroborates measurements of the physicochemical characteristics of the oils (Table 2). Rather, high RI values are an indication of the presence of considerable amounts of PUFA in the oils [26,27]. The high content of linoleic acid would increase the susceptibility of the oils to oxidation.

Tocopherol contents were also determined and each cultivar was found to contain appreciable amounts of these constituents (Table 4).The concentration of ã -tocopherols were found to be greater than the δ- and á-tocopherol which is quite different than mungbean.

Table 3: Fatty acid profile of mash seed oil

Fatty Acid	M1	M2	M3	M4
C16	11.31ns ± 2.20	10.99 ± 1.99	11.23 ± 1.87	12.09 ± 1.58
C18	2.09ns ± 0.63	2.70 ± 0.24	2.89 ± 0.43	2.17± 0.77
C22	0.99ns ± 0.14	1.00 ± 0.29	0.87 ± 0.22	0.93 ± 0.30
C18:1	26.62b ± 0.07	26.74a ± 0.15	27.34ab ± 0.25	26.65ab ± 0.35
C18:2	07.19ns ± 4.47	08.93 ± 5.11	07.08 ± 3.74	08.64 ± 3.87
C18:3	51.80ns ± 0.03	49.64 ± 0.06	50.59 ± 0.05	49.52 ± 0.09

Table 4: Tocopherol content profile from each cultivar

Tocopherols (mg/100g of oil)	M1	M2	M3	M4
α	3.04 ± 0.89	02.97 ± 0.55	03.49 ± 0.17	3.17 ± 0.34
γ	721.99 ± 12.23	724.11 ± 15.75	722.06 ± 16.30	719.89 ± 20.29
δ	16.94ns ± 3.31	16.78 ± 4.19	17.09 ± 2.66	17.15 ± 4.00

This is the first report that provides detailed characterization of black gram seed oil. In this study the antioxidant potential of black gram seed oil has been explored. Additionally, inter varietal difference with respect to antioxidant contents was also studied.

This study of black gram in Pakistan has demonstrated biochemical differences among the cultivar samples. Previously reported data provided information about physical and biochemical differences, among various cultivars however chemical aspects are insufficiently studied. We were also able to identify the percentage composition and we found that ã tocopherols were higher in percentage. It is becoming progressively more understandable that tocopherols demonstrate high anticancer activities. Because of rapidly accumulating reports addressing effective targeting of wide-ranging regulators of carcinogenesis by δ and ã tocopherols, these compounds are being tested extensively using in-vivo and in-vitro assays. It is getting increasingly notable that ã-T and δ-T are more readily side-chain degraded therefore, urinary levels of ã- and δ-short side chain metabolites may be explored as promising bio- markers for the consumption of ã-T and δ-T.

Conclusion

The results may therefore offer a scientific basis for use of the seeds, both in human diet and some commercial products and consumption of the black gram cultivars, in addition to providing such nutrients as proteins, carbohydrates, and minerals, also impart some of the widely acclaimed health benefits of the these oil constituents to the indigenous population of Pakistan. Given the continuing population growth and the still low per capita income of the indigenous population, the prospects for further expanding black gram utilization appear bright. To achieve this, however, priority needs to be given to black gram cultivation in national development programs and continued strong support for both basic and applied research on black gram is required at the national level to maintain the momentum generated by current improvements.

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