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Original Research Article

BASIC COMPOSITION AND CALORIC CONTENTS OF MACROTYLOMA UNIFLORUM (LAM.) VERDC., PHASEOLUS LUNATUS LINN., AND PHASEOLUS VULGARIS LINN., LEGUME FLOURS

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ABSTRACT

The % age basic composition (ash, crude fat, crude protein, moisture and total carbohydrates) were determined in the *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn., legume flours. *M. uniflorum* contained moisture (8.9), ash (3.34), crude fat (1.30), crude protein (18.15) and total carbohydrate (68.31). Similarly, moisture (9.14), ash (3.73), crude fat (1.78), crude protein (22.64) and total carbohydrate (62.71) were estimated in *P. lunatus.* Whereas, *P. vulgaris* contained moisture (9.43), ash (3.61), crude fat (3.49), crude protein (19.46) and total carbohydrate (64.01). The gross, ash free, moisture and ash free calorific values were also determined. *P. vulgaris* showed highest(3.91) and *P. lunatus* lowest (3.84), whereas of *M. uniflorum* (3.86) caloric values (kcal/g) respectively. Basic composition and caloric values of tested legume flours justify them as good sources of nutrition and energy.

Key Words: *Macrotyloma uniflorum*, *Phaseolus lunatus*, *Phaseolus vulgaris*, Carbohydrate, Protein, Fat, Calories.

INTRODUCTION

The estimation of seeds basic composition is necessary as they contain reserve food supplies and growth substances that influence seed germination and seedling vigor, seed storage and longevity. These are the basic and important source of food and medicine for man and animals[1]. Ash is obtained by herbaceous bio mass combustion. The contents of biomass ashes may originate from materials that the plant absorbed from the water or the soil during its growth, or from the soil collected along with biomass. Biomass ashes are rich in important plant nutrients such as Ca, K, Na, P and Si. As compare to herbaceous, woody biomass contain higher and lower concentrations of silica and calcium respectively[2]. Carbohydrate contents of beans comprise of total carbohydrate, soluble sugars and crude fiber. Total carbohydrates consist of starch and amylose. Total soluble sugars include ajugose, raffinose, stachyose, sucrose and verbascose. The crude fiber consists of lignin, cellulose and hemicellulose. Cellulose is the major component of crude fiber in *Phaseolus lunatus*, *Phaseolus vulgaris* and *Vigna unguiculata.* While in other legumes (*Cajanus cajan*, *Lupinus albus*, *Vicia faba* , *Vigna mungo*), hemicellulose is the major component of fiber[3] Legume seeds contain about 20-40% proteins (dry weight) and act as richest food sources for human and animal nutrition[4]. The main protein fractions are storage protein (albumin and globulin) used during the seed germination and development of plant. These storage proteins are about 70-80% of the crude protein. The enzymes (lipoxygenase and urease), enzyme inhibitors (trypsin inhibitors), hormones (gibberllins), transporting, structural and recognition proteins (lectins) behave as minor non-storage proteins[5]. Lipid contents act as food reservoir of plants and provide necessary energy for rapid growth and development. Higher lipid contents of plants reflected in higher caloric values. Caloric contents of different parts of plant are in following order: seeds > fruits > flowers > leaves > bark > roots [6]. Plant parts performing particular functions have high caloric values as seeds of reproductive parts. The plant parts producing no organic compounds maintain low caloric values[7]. Among vegetative parts, leaves contain high caloric values because of containing and composing high energy compounds proteins and fats. Other vegetative parts which acts as supporting organs (roots, stem and branches) contain cellulosic fibers, thus contain low caloric values[8]. Furthermore, the increasing altitude and a decrease in temperature, increases iodine number (fat) and ultimately caloric contents [6]. Leguminosae is the largest flowering plants family consists of 650 genera and 18,000 species. Legumes and cereals are providing food crops for world agriculture. Legume grains are considered as highly nutritious food resource and health protecting compounds. The developing and under developed countries largely consume grain legumes to fulfill their nutritional requirements and consider as "the poor man's meat"[4,5]. Due to having these facts legume grains were selected for current study. The basic composition of *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn., legume flours as well as the caloric contents were determined in the present study.

MATERIALS AND METHODS

Plant material identification and sample preparation

Beans of *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn., were purchased and identified by a taxonomist Department of Botany, University of Karachi. The voucher specimen number of *Macrotyloma uniflorum* (Lam.) Verdc., (G.H.No.86483), *Phaseolus lunatus* Linn., (G.H.No. 86451) and *Phaseolus vulgaris* Linn., (G.H.No. 86536) were deposited in the Herbarium of University of Karachi. The seeds were separately grinded and powdered then passed through 600µm sieve and kept in an amber bottle at room temperature before commencing the experiment.

Apparatus and Instruments

Hot air oven Model E 28 (Binder, Germany), oxygen bomb calorimeter Model No. PARR-1281 (Parr instrument company, USA), soxhlet apparatus (extractor soxhlet with Allihn condenser and flask, Wert lab Germany) and heating mantle (MTOPS, Korea).

Chemicals and Reagents used

Hydrochloric acid, mercury(II) oxide, methyl red, petroleum ether, potassium sulfate, sodium hydroxide, sodium thiosulfate pentahydrate solution, sulfuric acid (Merck, Germany).

Analytical Methods

Ash: The AOAC Method 923.03 was used to determine ash contents[9]. The 2g of tested legume flours were ignite separately at 550°C and weighed the obtaining light gray ash. The % ash was calculated by using following formulae.

$$\text{Ash \%} = \frac{\text{Weight of test portion} - \text{Weight loss on ashing}}{\text{Weight of test portion}} \times 100$$

Moisture: The moisture content was analyzed by using AOAC Method 925.[10]. To obtain moisture content, 2g of tested legume flours were placed for 1h in previously heated hot air oven maintained at 130±3°C. The moisture% was calculated by using following formula.

$$\text{Moisture \%} = \frac{\text{Difference in sample weight}}{\text{Weight of sample}} \times 100$$

Crude Fat: The fat content of tested legume flours determined by AOAC Method 945.38. The 2g of tested legume flours were extracted separately by using petroleum ether for 4-6h in soxhlet apparatus. The weight of extracted fat was taken after drying at 100°C for 30 mins. The % age fat was determined by following formula.

$$\text{Crude fat \%} = \frac{\text{Extracted fat}}{\text{Weight of sample}} \times 100$$

Crude Protein: The crude protein in legume flours was determined by using AOAC Method 920.87 (based on Kjeldahl digestion and Titrimetry)[9]. The 2g of sample was digested in 25ml of conc. sulfuric acid in the presence of 15g powdered K_2SO_4 and 0.7g of HgO as catalysts, to convert the amine nitrogen to ammonium ions. The solution was boiled briskly for 2h till it become clear and free from undigested material. Distillation of diluted digest was done by using 50ml HCl, 25ml thiosulphate solution (8%) and 75ml of NaOH (45%) solution. The diluted digest was heated until all ammonia has been distilled. In this regard, ammonia (nitrogen) was separated from the digestion mixture. Titration of HCl receiving distillate was done against NaOH solution using methyl red indicator. In this way, the trapped ammonia was determined by titration. The % nitrogen (N) later on crude protein was calculated by following formula.

$$\text{Nitrogen\%} = \frac{(\text{ml std. acid} \times \text{morality of acid}) - (\text{ml std. NaOH} \times \text{molarity of NaOH}) \times 1.4007}{\text{Weight of test portion (g)}}$$

$$\text{Crude protein} = \text{Nitrogen\%} \times 6.25$$

Total Carbohydrates: The total and available carbohydrate was calculated by following formula.

$$\text{Total carbohydrate} = 100 - (\text{ash\%} + \text{moisture\%} + \text{crude fat\%} + \text{crude protein\%})$$

$$\text{Available carbohydrate} = 100 - (\text{ash\%} + \text{moisture\%} + \text{crude fat\%} + \text{crude protein\%} - \text{dietary fiber\%})$$

Calorie: The caloric content of tested legume flours were analyzed by oxygen bomb calorimeter and caloric values were calculated by using ASTM standards[10].

RESULTS AND DISCUSSION

The results of the present study are shown in table-1, 2 and figure-1, 2. Ash, moisture, crude fat, protein and total carbohydrates were analyzed by AOAC official methods. The ash content (%age) was estimated as *Macrotyloma uniflorum*(3.34), *Phaseolus lunatus* (3.73) and *Phaseolus vulgaris* (3.61). The ash content and composition depend on the part of plant and growing conditions. Leaves contain higher amounts of ash than stems. Si and other inorganic elements uptake in the plant is the response of water uptake. Fertilizers play an important role in the variation of chlorine, phosphorus and potassium contents in herbaceous ash. So, recycling of herbaceous biomass also behave itself as a fertilizer [2]. The % moisture content of *Macrotyloma uniflorum*, *Phaseolus lunatus* and *Phaseolus vulgaris* legume flour was 8.9, 9.14, 9.43 respectively. During growth and storage, temperature and relative humidity are environmental factors affecting moisture content. Metabolic water produced during storage is another factor affecting moisture content[11].

The crude fat contents were found as *M. uniflorum*(1.30%), *P. lunatus* (1.78%) and *P. vulgaris* (3.49%). *M. uniflorum, P. lunatus* and *P. vulgaris* contain 18.15, 22.64 and 19.46% crude protein contents. The protein content of cooked legume seed and bread (70-100 g/kg cooked food) is similar, but still much higher than potato (15-22 g/kg). Legume proteins as compared to cereals are rich in lysine and poor source of sulfur-containing amino acids (methionine and cysteine)[5]. Legume proteins have beneficial role against cancer, cardio-vascular diseases, diabetes, immunity-related diseases and obesity[4].The crude protein in legume flours was estimated by using determined nitrogen content and nitrogen-to-protein conversion factor (Jones' factor) 6.25 as the standard[9]. The estimated carbohydrate contents were 68.31% in *Macrotyloma uniflorum*, 62.71% in *Phaseolus lunatus* and 64.01% in *Phaseolus vulgaris*. Legume carbohydrates, are beneficial in the management of diabetes and hyperlipidemia as their property of 'slow release carbohydrate[12]. There are four procedures to evaluate calories. In component analysis, the separate analysis of ash, moisture, crude fat, protein and total carbohydrates and the summation of these values suggested as caloric value. Wet oxidation assumes that in all organic matter the carbon present is carbohydrate. This method is unable to give true oxidation of carbon. Thermochemical method assumes that all organic compounds are intermediate in oxidation state between CH_4 and CO_2. Rearrangement of the inter-atomic bonds is responsible for energy liberation in oxidation. This method allows about 1% accuracy. The fourth and widely used method is calories /g from bomb calorimeters. It is considered to be an accurate method for calorie estimation. It gives better and easily obtained caloric values by direct combustion of material. Its ease (small sample size) and rapidity (reduced time for sample combustion) make it preferable[13]. The USDA reported calories in whole grains of *P. lunatus* as 3.38kcal/g [14] and in *P. vulgaris* as 3.37kcal/g[15]. Whereas data of *M. uniflorum* was not found in USDA National Nutrient Database. The estimated caloric values (kcal/g) of *P. lunatus* (3.84) and *P. vulgaris* (3.91) are greater than USDA reported values showing comparable good source of energy.

The direct estimation of calorific values from samples known as gross calorific values (GSV). When ash free samples are used to determine caloric values is known as ash free calorific value (AFCV). Whereas, moisture and ash free calorific values (MAFCV) are determined by using moisture and ash free sample. *P. vulgaris* contain highest GCV(3.91), AFCV(4.37) and MAFCV(4.22) kcal/g. The estimated GCV, AFCV and MAFCV in *P. lunatus* are 3.84,4.33 and 4.17 and in *M. uniflorum* are 3.86, 4.31 and 4.19. Ash free caloric values preferably be used in place of gross caloric value because ash greater than 10% creates error in caloric estimation due to the decomposition of calcium carbonates and other salts in ash at high temperature of bomb calorimeter[16]. The tested legume flours of *Macrotyloma uniflorum, Phaseolus lunatus* and *Phaseolus vulgaris* contain 3.34-3.73% ash content (less than 10%). Here we determined both GCV and AFCV to avoid chances of error and comparison in between them. From obtaining results, it has declared that AFCV estimation is preferable to obtain correct caloric value. The comparison of AFCV and MAFCV shows that moisture content has direct effect on caloric value. The presence of considerable ash, moisture, crude fat, protein and total carbohydrates also showing the basis for high calorific values of tested flours[13]. Calorie needs depends on age, weight and activity level. USDA estimates that majority of men need 2.0 - 3.0 and women (excluding pregnant or breastfeeding) 1.6 - 2.4 kcal/day to maintain health. Active men and athletes may require about 3.0 kcal/day to maintain a healthy weight[17]. Overweight men and women require 1.2 - 1.6 and 1.0 - 1.6 kcal/day respectively[18]. Table-3 showing USDA reported caloric values of different foods. The caloric values (kcal / g) of *Macrotyloma uniflorum* (3.86), *Phaseolus lunatus* (3.84) and *Phaseolus vulgaris* (3.91) are less than *Glycine max* (4.46) and nuts such as *Anacardium occidentale* (5.53), *Arachis hypogaea* (5.67), *Carya illinoinensis* (6.91), *Juglans regia* (6.54), *Macadamia integrifolia* (7.18), *Pinus gerardiana* (6.73), *Pistacia vera* (5.60) and *Prunus dulcis*(5.79). But, when comparing with calorie needs / day these caloric values are sufficient to fulfill body demands of men, women even active men and athletes.

CONCLUSION

From the results, it may be concluded that all three legumes *Macrotyloma uniflorum*, *Phaseolus lunatus* and *Phaseolus vulgaris* contain appreciable amount of ash, moisture, crude fat, protein and total carbohydrate and as a result high caloric value comparable to nuts. Therefore, it may be said that these legumes are the good source of nutrition and energy and should be a part of our daily food.

Table.1. Basic composition of *Macrotyloma uniflorum, Phaseolus lunatus* and *Phaseolus vulgaris* legume flour.

Tested samples	% composition						
	Moisture	Ash	Crude fat	Crude protein (N x 6.25)	Total carbohydrate	Available carbohydrate	Total dietary fiber[19]
M. uniflorum	8.90± 0.09***	3.34± 0.01	1.30±0.02**	18.15±0.01***	68.31±0.00***	83.90±0.02***	15.59
P. lunatus	9.14± 0.01***	3.73± 0.01	1.78±0.02*	22.64±0.01***	62.71±0.00***	74.71±0.01***	12.00
P. vulgaris	9.43± 0.00***	3.61± 0.01	3.49±0.02	19.46±0.01***	64.01±0.04***	73.09±0.03***	9.08

Each value is a mean of five different determination ± S.E.M.; *p<0.01; **p<0.05; ***p<0.001 vs. control showing significant, more and most significant values using unpaired Student's t-test.; reported values of total dietary fiber [19].

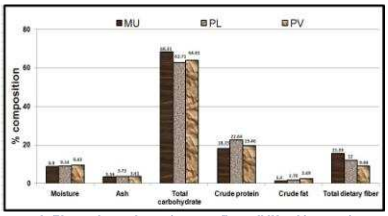

Figure 1 : Basic composition of *Macrotyloma uniflorum, Phaseolus lunatus* and *Phaseolus vulgaris* legume flour (MU= *Macrotyloma uniflorum*, PL= *Phaseolus lunatus*, PV= *Phaseolus vulgaris*).

Table 2: Different calorific values of *Macrotyloma uniflorum, Phaseolus lunatus* and *Phaseolus vulgaris* legume flour

Tested samples	Caloric values (kcal/g)		
	GCV	AFCV	MAFCV
M. uniflorum	3.86±0.00**	4.31±0.02*	4.19±0.04*
P. lunatus	3.84±0.00**	4.33±0.02*	4.17±0.02*
P. vulgaris	3.91±0.00**	4.37±0.00*	4.22±0.02*

GCV=Gross Calorific Value; AFCV= Ash Free Calorific Value; MAFCV= Moisture and Ash Free Calorific Value; Each value is a mean of three different determination ± S.E.M. *p<0.01; **p<0.05 vs. control showing significant and most significant values using unpaired student's t-test.

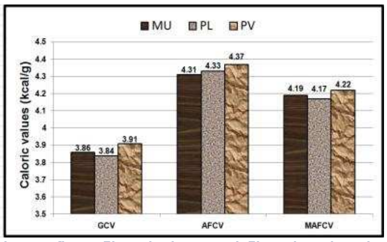

Figure 2: Comparison between GCV, AFCV and MAFCVof *Macrotyloma uniflorum,Phaseolus lunatus* and *Phaseolus vulgaris* legume flour (MU= *Macrotyloma uniflorum*, PL= *Phaseolus lunatus*, PV= *Phaseolus vulgaris*).

Table 3: USDA reported caloric values (kcal/g) of foods[20]

Foods		
Latin name	**Common name**	**Caloric values as kcal/g***
	Fruits	
Ficus carica	Fig	1.07
Malus domestica	Apples with skin	0.52
Mangifera indica	Mango	0.60
Musa acuminata	Banana	0.89
Persea americana	Avocado	1.60
Phoenix dactylifera	Dates	2.82
Punica granatum	Pomegranate	0.83
Vitis vinifera	Raisins	2.99
	Grapes	0.67
	Legumes, cereal	
Cajanus cajan	Pigeon pea	3.43
Cicer arietinum	Chickpeas	3.78
Glycine max	Soybeans	4.46
Lens culinaris	Lentils	3.52
Triticum aestivum	Wheat	3.39
Oryza sativa	Rice, white	3.65
Vigna radiata	Mung beans	3.47
Vigna unguiculata	Cowpeas	3.36
Zea mays	Corn grain, yellow	3.65
	Nuts	
Anacardium occidentale	Cashew nuts	5.53
Arachis hypogaea	Peanuts	5.67
Carya illinoinensis	Pecans	6.91
Juglans regia	Walnuts	6.54
Macadamia integrifolia	Macadamia nuts	7.18
Pistacia vera	Pistachio	5.60
Pinus gerardiana	Chilgoza pine	6.73
Prunus dulcis	Sweet almond	5.79
	Sea foods	
Paralithodes camtschaticus	Crabs, king	0.97
Chionoecetes opilio	Crabs,queen	1.15
Nephropidae	Lobster	0.89
Salmo salar	Salmon fish	1.78
Penaeus orientalis	Shrimp	0.99
	Vegetables	
Beta vulgaris	Beet root	0.43
Daucus carota	Carrot	0.41
Ipomoea batatas	Sweet potato	0.86
Solanum tuberosum	Potato	0.77

*Caloric values of unprocessed mature fruits, legumes, nuts, vegetables and cooked sea foods.

CONFLICT OF INTEREST

Authors Declare no Conflict of Interest.

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