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TOTAL, INSOLUBLE AND SOLUBLE DIETARY FIBER CONTENTS OF MACROTYLOMA UNIFLORUM (LAM.) VERDC., PHASEOLUS LUNATUS LINN., AND PHASEOLUS VULGARIS LINN., LEGUME FLOURS

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ABSTRACT

Current study was conducted to determine the insoluble, soluble and total dietary fiber content of *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn., legume flours by using AOAC enzymatic - gravimetric analysis. The results showed that *M. uniflorum*, *P. lunatus* and *P. vulgaris* legume flours contained 155.91,120.01 and 90.80 mg/g insoluble dietary fiber, respectively. Whereas, soluble dietary fiber was not found in any tested sample.

KEYWORDS: *Macrotyloma uniflorum*, *Phaseolus lunatus*, *Phaseolus vulgaris*, Dietary fiber, AOAC enzymatic - gravimetric analysis.

INTRODUCTION

Dietary fiber (DF), an important component of human nutrition is a complex mixture of non-starch polysaccharides together with cellulose, fatty acids, gums, hemicellulose, lignin, mucilages, pectins, protein and waxes[1]. The American Association of Cereal Chemists (AACC) define dietary fiber as "Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation,

and/or blood cholesterol attenuation, and/or blood glucose attenuation[2].” Dietary fiber is responsible for delaying stomach emptying, speeding gut transit, binding minerals, changing enterohepatic circulation, altering the rate of mucosal cell proliferation, contributing to the nutrient supply and modifying gut hormone release specially enteroglucagon, gastro inhibitory polypeptide and somatostatin[3]. Dietary fiber is divided into Insoluble (IDF) nonviscous/slowly fermentable and soluble (SDF) /viscous/fermentable[1]. SDF includes pectin and gums. It regulates digestion and absorption in the small intestine. Its viscous nature help to treats cardiovascular diseases by lowering blood cholesterol levels. By normalizing blood glucose and insulin levels it help to treat type 2 diabetes[4]. SDF binds with bile acids, increasing their fecal excretion and interrupting the enterohepatic circulation of bile salts ultimately reduce fat absorption and thus contributing hypolipidemic effects. SDF extensively fermented by anaerobic bacteria in the human colon and the fermentative end products of fiber are short chain fatty acids such as acetate, propionate, and butyrate. These short chain fatty acids are absorbed and contribute to the hypolipidemic and glycemic effect[5]. Propionic acid and acetic acid are metabolized in the liver, except butyric acid which is used locally as an essential source of energy, by the gut colonocytes. The multiplication of the bacterial flora increases the bulk and the water content of the stools. Bile acid and divalent cations, such as calcium, zinc and iron, are bound to the dietary fiber in small intestine and thus reduces their absorption[6].A fiber-rich meal is less caloric, lower in fat which is characteristic to treat and prevent obesity[4]. SDF protects from colon cancer due to its antioxidant functions[5]. In large intestine, IDF effectively increase fecal volume, causes rapid peristaltic contraction and act against constipation[7]. As a result of slow fermentation by micro flora in the large intestine IDF also prevents from diverticulosis and diverticulitis[4]. In view of their health benefits, WHO recommended an increase in the dietary fiber daily consumption. For these reasons, wide variety of food items have been analyzed for their dietary fiber content, because of their health benefits[8]. Besides, the nutritional importance, DF also has desirable functional properties to provide texture, gelling, thickening, emulsification, and stabilization in foods. Therefore, DF research has drawn much attention in the nutraceutical industries[9]**.** Historically, crude fiber analysis was developed to measure indigestible material in animal feed. It just measures small fraction of total dietary fiber as all soluble polysaccharides become lost. The colorimetric and gas chromatographic assay for dietary fiber estimation are complex and too laborious. Gravimetric methods are divided into neutral detergent and acid detergent fiber analysis (Van Soest´s method) and enzymatic - gravimetric analysis (Prosky method or its modification). Van Soest´s method consists of the

gravimetric determination of residue previously treated with acid and neutral detergent solutions. Only insoluble dietary fiber components are determined by this method. Enzymatic - gravimetric analysis is the simpler, faster and satisfactory method uses amylolytic and/or proteolytic enzymes to determine both IDF and SDF[10-12]. Legumes are an excellent source of protein, carbohydrates, fiber, minerals, and other nutrients. Legumes as economical nutritional source, play an important role in the diets of developing countries population[13]. Attention has been focused on the dietary fiber content of legumes. Current study was an attempt to estimate TDF, IDF and SDF of *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn., legume flours by using AOAC enzymatic - gravimetric analysis.

MATERIALS AND METHODS

Chemicals and Reagents used

Acetone, ethanol, hydrochloric acid, petroleum ether, sodium hydroxide (Merck, Germany); amyloglucosidase (Sigma No. A9913), α-amylase (Sigma No. A3306) and papain (Sigma No. 1495005) (Sigma-Aldrich Chemie, Switzerland).

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 after defatting.

Determination of TDF, IDF and SDF

The raw legume samples were analyzed by Prosky method[14] for IDF and SDF according to AOAC Method 993.19 and 991.42, an enzymatic-gravimetric procedure[15]. Blank was run along with samples to measure any contribution from reagents to residue. Defatting of *M. uniflorum*, *P. lunatus* and *P. vulgaris* seeds have done with 25ml of petroleum ether / g of sample three times to remove fixed oil. Then, legume flour was prepared through milling. Residual moisture was determined in milled samples by drying for 5h at 105°C in hot air oven. Weigh duplicate 1gm tested sample. Phosphate buffer (pH 6.0, 50 mL) was added to sample. Adjust to pH 6.0 ± 0.2 by adding 0.3N NaOH or 0.3N HCl. Enzyme hydrolysis of sample was started by adding 0.1mL α-amylase, incubate at 95 – 100 °C for 30 minutes in

water bath with continuous agitation. Cool to room temperature. Adjust to pH 7.5 ± 0.2 by adding 10ml of 0.3N NaOH. Papain 5mg was added, incubate at 60 °C for 30 minutes in water bath with continuous agitation. Cool to room temperature. The pH was adjusted to 4.0 – 4.6 by adding 10ml 0.3N HCl. Amyloglucosidase 0.3mL was added, incubate at 60 °C for 30 minutes let precipitates to form and filter it. Weigh the residue. In case of soluble fibers, filtrates plus washing were mixed with 400mL of 95% ethanol to precipitate materials that were soluble in the digestates. After 1h, precipitates were filtered. Residue was washed successively three times with 20mL of 78% ethanol and two times with 10mL of 95% ethanol and then acetone respectively. For insoluble dietary fiber estimation, residue was washed with 10mL of water (for removing soluble dietary fibers), 95% ethanol and then acetone respectively. Residue was dried at 105°C for 5h in hot air oven, cool in dessicator and weigh to 0.1mg separately (S_1 and S_2) . The S_1 and S_2 were used for ash and protein estimation respectively. For ash determination S_1 was incinerated at 525 °C for 5h in hot air oven. The N x 6.25 conversion factor was used to analyze protein in S_1. The soluble and insoluble dietary fibers (%) were calculated by using following formula.

SDF or IDF, % = [(residue – protein – ash – blank)* / weight of test portion] x 100
TDF, %= SDF + IDF
Where,
SDF= soluble dietary fiber
IDF= insoluble dietary fiber
TDF= total dietary fiber
* indicates values taken as mg of weight
Weight residue = average of duplicate
Weight of test portion = average of duplicate

Statistical analysis. The values were expressed as means ± standard deviations. As in all tested samples there was no SDF. So, IDF (mg/g) were subjected to unpaired student's *t*-test. All statistical calculations were performed with SPSS-20.

Table 1: Fiber contents (mg/g) of *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* Linn., and *Phaseolus vulgaris* Linn., legume flour.

Tested samples	IDF*	IDF as % TDF
M. uniflorum	155.91±0.04*	15.59
P. lunatus	120.01±0.02*	12.00
P. vulgaris	90.80±0.00*	9.08
TDF= total dietary fiber; IDF = insoluble dietary fiber; N=3 duplicate determinations for samples; values taken as mg/g. Results are mean of values ± S.E.M.); S.E.M.=Standard Error of Mean; *P<0.05 showing significant values using unpaired student's *t*-test.		

RESULT AND DISCUSSION

The IDF of *M. uniflorum*, *P. lunatus* and *P. vulgaris* legume flours are estimated as 155.91, 120.01 and 90.80 mg/g respectively**(table-1)**. SDF have already been reported from the same samples. But SDF was not detected in any tested sample. The already reported SDF which are in lowest quantity, may be lost during milling process. Therefore, in this case IDF is equal to TDF. Previous studies on *M. uniflorum* legume flour by using AOAC enzymatic-gravimetric method reported 216.10(IDF), 8.60(SDF) and 224.70(TDF) in mg/g[16], whereas, in *P. vulgaris* 171 (IDF), 77 (SDF) and 245 (TDF) mg/g[17]. Our results are comparable with the literature data, although the previously studied flours showed significantly higher values of IDF, TDF and the presence of SDF. Although the dietary fiber of *M. uniflorum* and *P. vulgaris* legume flour has already been estimated by using AOAC enzymatic gravimetric analysis. But variations in the obtaining data of dietary fiber by using same method for same tested material exist, these variations could be regional (soil and climatic) and genotypic[8]. Whereas, *P. lunatus* legume flour estimated for the first time. AOAC enzymatic - gravimetric method was recommended by the U.S. Food and Drug Administration to determine DF. Estimation of DF through enzymatic–gravimetric method is consider to be accurate, precise and reliable[18]. Therefore, the same AOAC method was selected for dietary fiber estimation. α-amylase was used to gelatinize the sample, papain as protease to remove protein. Whereas, amyloglucosidase was used to remove the starch from the sample[15]. The USDA reported TDF in whole grains of *P. lunatus* as 190mg/g [19] and in *P. vulgaris* as 152mg/g[20]. Whereas data of *M. uniflorum* was not found in USDA National Nutrient Database. Milling of cereal or legumes reduces dietary fiber contents[21]. Therefore, estimated TDF values are lower than USDA reports. Daily Reference Intakes (DRI) has been developed, since 1996 by the Food

and Nutrition Board, Commission on Life Sciences, National Research Council, to replace the Recommended Dietary Allowance (RDA). Dietary Reference Intakes (DRIs) of dietary fiber (g/day) for children (1-13 yrs) is 19-31 and for adults male (14-50 yrs) is 38. Whereas, for adult women (14-50 yrs) is 25-26. The DRI increases as 28-29 (g/day) for the same age group pregnant or lactating women[22]. One cup (178g) of *P. lunatus* contain 33.8 g of dietary fiber[19]. Whereas, 28g of dietary fiber is present in a cup (184g) of *P. vulgaris*[20]. So, *P. lunatus* and *P. vulgaris* comply DRIs of dietary fiber (g/day) for all age groups and for both sexes. American Dietetic Association recommended the inclusion of dietary fiber by using variety of cereal, legumes, vegetables and fruits for an active and healthy life, as consumption of fibrous diet in developed countries is low[23]. Therefore, growing interest of dietary fiber increases consumption of legumes, cereals, fruits and seaweeds.

CONCLUSION

Therefore, from the results it may be concluded that, all three legumes *Macrotyloma uniflorum*, *Phaseolus lunatus* and *Phaseolus vulgaris* are the good source of dietary fibers specially IDF.

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