Journal of Biotechnology and Bioengineering
Volume 4, Issue 1, 2020, PP 14-19
ISSN 2637-5362

SDS-Page Protein Profiling of a Few Commonly Consumed Beans

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ABSTRACT

Beans are widely used to make delicacies and they are mainly comprised of protein. In this study, we compared the protein content of 7 types of commonly consumed beans, namely namely Arachis hypogaea (peanut), Hoedeum vulgare (barley), Vigna radiata (green bean), Vigna angularis (red bean), splits seeds of Pisum Sativum (golden gram) and Phaseolus vulgaris (black turtle beans). Each of the beans were ground into powder form and tris buffer was used to extract the proteins. Similar quantity of the beans' protein were respectively separated by SDS-PAGE. We found that black turtle beans contained the highest amount of protein, while golden gram has the highest variation of protein types. Barley was found to contain the least quantity of protein and protein types. The difference in protein profile of each of the beans indicate that although each of the beans are rich in protein, their nutritional value may not be the same and each has its own unique value to human health.

Keywords: *SDS-PAGE, beans, protein profiling*

INTRODUCTION

Beans are commonly used in both sweet and savoury dishes. They are often made into paste as filling for desserts or used in savoury soups (1). Protein made up most of the composition of beans. In this study, we have carried out comparison of the protein content between a few commonly use beans, namely *Arachis hypogaea* (peanut), *Hoedeum vulgare* (barley), *Vigna radiata* (green bean), *Vigna angularis* (red bean), splits seeds of *Pisum Sativum* (golden gram) and *Phaseolus vulgaris* (black turtle beans).

Peanut is edible in the forms of either cooked, roasted or raw. Peanut is technically legumes, therefore its nutritional profile is closer to other expensive nuts such as almonds, pistachios and macadamias (2). The nutrient value of peanut is mostly due to its fatty acid composition (3), which was said to lower total blood cholesterol (TC) as well as low-density lipoprotein (LDL) levels and increases high-density lipoprotein (HDL).The unsaturated fatty acids in peanuts is also known to provide cardio protective effect(4-7). Besides, it is found to have antidiabetic activity (8), and platelet aggregation inhibitory effect (9).Protein storage in peanut from vicilin, conglutin and glycinin families at MW 14-65 kD (10)are often known as allergen in peanuts. Approximately 10% of peanut proteins are albumin while the other 90% proteins are anionic globulins with two major fractions, arachin and conarachin.

Barley is commonly cooked into drink or served in the form of dessert. It is well-known for its cooling effect. Barley is traditionally used as anti-inflammatory agent and cardiovascular protectant (11),to treat haemorrhoid and inflamed wounds (12) and anti-inflammation (13, 14).Crude protein found in barley is 11.84% dry basis (15). Amino acids found from barley include arginine, histidine, lysine, tyrosine, tryptophane, phenylalanine, cystine, methionine, threonine and leucine (16).

Green beans or mung beans are popular ingredient for dessert preparation. It is often used in sweet soups, made into a paste, or ground into flour for cookies or noodles (1). Green bean is used for its detoxification activities as well as alleviation of heat stroke. It is also used for gastrointestinal regulation and skin moisturizing (17),activity against *Helicobacter pylori*(18) and anti-diabetic effect(19). Studies showed that high levels of protein, polyphenols, oligosaccharides and amino acids in green beans greatly contribute to its antimicrobial, anti-inflammatory, antioxidant, antitumor and lipid metabolism regulation activity (18, 20-22).

Red bean is one of the most commonly used beans in dessert. It is used in steam bun, bread and variety of desserts (1). It has been traditionally used by the Chinese to treat diarrhoea, vomiting, edema and other diseases(23). Red bean is rich in polyphenols which give rise to its strong antioxidant activity. Proteins such as albumins, globulins, prolamin, and glutelins were extracted from red beans, these proteins contributing to antihypertensive activity, antiradical activity, as well as anti-chelating activity(24).

Golden gram also known as yellow splits peas are commonly use in Indian cuisine. It is rich in carbohydrates, fibre, vitamin C, protein and vitamin B(25). Golden gram was found to have both vicilin and albumin fractions. Golden grams is used traditionally by Indian community for wound healing, measles, alleviating burnt skin (26), to improve appetite, act as laxative and cough suppressant (27). The phenolic content are responsible for its anti-oxidant property which is also anti-cancer effect(28).

Black turtle bean is also known as black bean and it is a common ingredient in Indian dishes. Black turtle bean is pre-soaked in water before it is cooked. It is normally made into dal, curry, soup, sandwich filling, burger patties, masala and salad(29). Studies showed that the polyphenolics content in black turtle bean could lower the incident of colon cancer(30, 31). Anthocyanins content in black turtle beans has high anti oxidative activity(32, 33).

Major components of beans are protein, and in view of the beneficially effects of beans on human health, it will be valuable to study the protein content of beans that are commonly consumed in Malaysia, we hope that the data collected from this study may contribute to the knowledge of nutritional usages of beans.

METHODOLOGY

Protein Extraction

Beans with skin, including peanut, green bean, red bean and black turtle bean were soaked in distilled water until the skin were soften and removable. The beans without skin like barley, green gram and golden gram were rinsed with distilled water to remove impurities. All beans were dried in oven with temperature of 27℃ for overnight. Dry weight of the beans was obtained when there was no significant changes in the weight.

The dried beans were grinded into powder for protein extraction. Tris buffer (40mM) was used to extract protein from the beans. A ratio of weight of beans to the volume of tris buffer, ratio 1:4 was used (except for barley, 1:1.5 ration was used). The mixtures were vortex thoroughly and centrifuged at the speed of 13,000 rpm for 30 minutes in 4℃. The clear supernatant was recovered.

SDS-PAGE

The method of SDS-PAGE analysis was carried out based on the procedure described by Leammli (1970).A 10% resolving gel and 4% stacking gel were prepared. Protein extract was diluted 2 times and the ration of extract to reduced sample buffer was 4:1. For mini gel, 20µL of each mixture was loaded in duplicate and run under constant 160V for 1 hour. As for the huge gel format, 30µL were loaded to the well in duplicate and the samples were ran at constant 200V for 2.5 hours. The gels were stained with Commassie blue staining.

Protein Assay

The protein concentration was determined using RCDC (reducing agent, detergent compatible) Protein Assay Kit (Bio-Rad, USA). The analysis was carried out according to the guideline given by the manufacturer.

RESULTS

Table 1 shows the difference in the weight of beans before and after drying. Cleaning of the beans in water has caused the beans to absorbed some content of water, the beans after cleaning were subjected to drying process, and the beans were considered fully dried when there was no changes in the weight after a certain period of oven drying.

Table1. *Raw data for dry weight for all beans*

Type of Pea	Weight before drying (g)	Reading after drying 1 (g)	Reading after drying 2 (g)	Reading after drying 3 (g)	Reading after driying 4 (g)	Difference weight before and after drying	Percentage of changes (%)
Green bean	1.3206	1.3178	1.3038	1.2765	1.2831	0.0066	0.523
Red bean	5.4002	5.3807	5.3249	5.1883	5.2222	0.0339	0.687
Green Gram	37.6103	37.3905	37.1089	36.1683	36.1996	0.0313	0.117

Golden Gram	19.3945	19.3122	19.2525	18.9335	18.8466	0.0869	0.545
Barley	34.003	33.8756	33.5239	32.7502	32.8876	0.1374	0.556
Peanut	21.9885	21.9259	-	-	-	0.0626	0.285
Black Pea	5.1416	5.1189	-	-	-	0.0227	0.443

Table 2 shows the total protein concentration for all the beans tested. All the beans except barley were diluted 10 times before protein assay due to their high protein content, nevertheless, barley was presented with the least protein content, where barley was diluted 1.5 times for protein assay. It should be noted that increase in dilution factor may result in greater chance of deviation in the protein assay's reading as more interferences were removed. Black turtle bean was recorded with the highest protein concentration while barley was with the lowest protein concentration for the equal volume of pea solution.

Table2. *Result of RCDC protein assay*

Type of peas	Dilution Factor	Mg protein/ml Actual protein concentation
Golden Gram	10	10.744
Black turtle bean	10	14.669
Red Bean	10	12.836
Peanut	10	14.410
Green Gram	10	9.768
Green Bean	10	14.634
Barley	1	1.337

DIAGRAM 1 shows the result of large format gel SDS-PAGE electrophoresis loaded with standardised 25µg of protein. Protein ladder was also loaded as molecular weight reference. Each sample was loaded in duplicates to access the result consistency. The dotted lines to indicate the range of proteins' molecular weights as referenced to the protein ladder markers. All the beans except for barley have proteins with different molecular weight distributed throughout the gel, as for barley, most of the proteins were found at lower molecular weights.

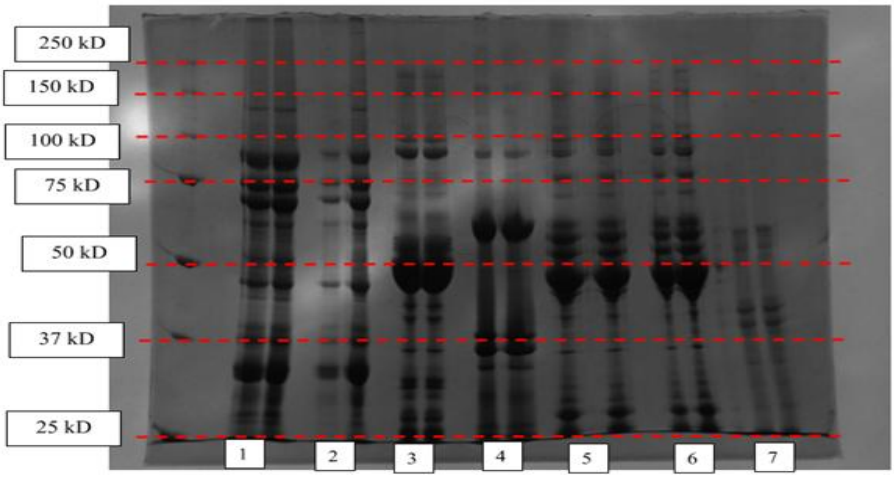

Diagram1. *Protein ladder reference relative to beans' protein.1: Golden gram, 2: Black turtle bean, 3: Red bean, 4: Peanut, 5: Green Gram, 6: Green bean, 7: Barley*

DISCUSSION

Beans are heavily used as delegacies all over the world, large portion of beans content are made up of proteins, therefore beans provide nutritional value to our diet. In order to study the protein content of the beans, the seed coat of the beans were removed by soaking in water. The beans were then dried in the oven till no changes in the beans dried weight (Table 1). The difference between the weight before and after drying indicates the water absorption capacity of the beans, when compare between the 7 beans tested, red beans was showed to have the highest water absorption capacity while green gram was with the lowest water absorption capacity. This water uptake ability may explain the shorter cooking time for red beans as compared with the green gram.

Black turtle bean has the highest amount of protein content, followed by green bean, peanut,

red bean, golden gram, green gram and barley. This is contraindicated by the results shown in United State National Nutrient Database, where barley was shown to be with higher protein content than black turtle bean while peanut was reported to have the highest protein content among the beans. The deviation may be due to the extraction method used or the source of the beans.

The total protein content for barley is relatively lower than other types of beans, barley contains much little protein content as compared to other beans per equal weight of bean. In this study, a similar quantity of protein was used for SDS-PAGE analysis. We found that similar quantity of protein will give a better ground for comparison study between beans. The optimum amount of protein that give an average band sharpness and better gel resolution was found to be at 25µg.

SDS-PAGE is a method to separate protein based on the protein size (34). Protein molecular weight can be estimated using this method. This technique can be used to separate protein with relative molecular not smaller than 10kD. Smaller size protein has lower affinity biding to SDS and it is not suitable to be separated using basic Leammli SDS-PAGE method (35).

Although most of abundant proteins found in all the beans were within the range of 37 to 75 kD, the electrophoretic migration of the abundant bands differs from bean to bean. This indicated that different proteins type was found in each bean. As refer to Diagram 1,lane five and six were green gram and green bean, respectively. The band patterns of these two beans were exactly the same. This is because they are the same species. However, they are sold in different forms, green bean is sold in the form with the seed coating, while green gram is sold without seed coating. The result from protein assay showed that green bean, the one with seed coating consist higher protein amount than green gram, the one without seed coating. In fact, the seed coating could preserve the protein contain in the seed by minimizing the protein loss. Golden gram appeared to have the greatest number of protein bands. This could imply it contains the most variety of proteins. Although black turtle beans showed highest protein quantity, it has the least protein band in SDS-PAGE, which indicates it has least type of protein compared to other beans. Although protein variety of black turtle bean is less, each type of proteins were presented in high quantity. As for barley, even though the protein loaded had been standardized with other beans, there are no band presented with higher abundance.

In general, each bean have their own unique protein profile. The total protein content in each beans does not proportionally correlate to the variety of protein contained in the beans. Although all the beans are of seed origins, the different in the protein profile for each beans indicate that they are made up of different protein types and each may have its own unique nutritional value needed by our body. The total protein content for barley is relatively lower than other types of beans. In this study, a similar quantity of protein (data obtained from protein assay) was used for SDS-PAGE analysis. We found that similar quantity of protein will give a better ground for comparison study between beans.

Conclusion

Barley has the lowest protein quantity and the black turtle beans was having the highest protein quantity for the equal dried weight of beans. Golden gram was having the greatest variety of protein in among the beans. Green bean and green gram are containing the same type of proteins due to their similar species. The difference in the protein profile of each beans indicated that although they are all comprised of proteins, the nutritional value of each bean are differ from one another.

References

[1] Kitchen MC. Beans and Seeds 2019 [cited 2019 6 March]. Available from: https://www.Malaysianchinesekitchen.com/ingredients/beans-and-seeds/.

[2] Fraisse JM. Nuts Over Peanut Butter. Star 2. 2015.

[3] Sheppard A, Rudolf T. Analysis of peanuts and peanut products for total lipids, fatty acids and proximates. Peanut Science. 1991;18(1):51-4.

[4] General USPHSOotS. The Surgeon General's report on nutrition and health: US Dept. of Health and Human Services, Public Health Service; 1988.

[5] Curb JD, Wergowske G, Dobbs JC, Abbott RD, Huang B. Serum Lipid Effects of a High–Monounsaturated Fat Diet Based on Macadamia Nuts. Archives of Internal Medicine. 2000; 160(8):1154-8.

[6] Garg ML, Blake RJ, Wills RB, Clayton EH. Macadamia nut consumption modulates favourably risk factors for coronary artery disease in hypercholesterolemic subjects. Lipids. 2007; 42(6):583-7.

[7] Gebauer SK, West SG, Kay CD, Alaupovic P, Bagshaw D, Kris-Etherton PM. Effects of pistachios on cardiovascular disease risk factors and potential mechanisms of action: a dose-response study. The American journal of clinical nutrition. 2008;88(3):651-9.

[8] Broadhurst CL, Polansky MM, Anderson RA. Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vitro. Journal of Agricultural and Food Chemistry. 2000; 48(3):849-52.

[9] Tang G-Y, Li X-J, Zhang H-Y. Antidiabetic components contained in vegetables and legumes. Molecules. 2008; 13(5):1189-94.

[10] Burks W, Sampson HA, Bannon GA. Peanut allergens. Allergy. 1998; 53(8):725-30.

[11] Khare CP. Indian medicinal plants: an illustrated dictionary: Springer Science & Business Media; 2008.

[12] Yeşil Y, Akalın E. Folk medicinal plants in Kürecik area (Akçadağ/Malatya Turkey). Turkish Journal of Pharmaceutical Sciences. 2009;6(3):207-20.

[13] Gul S, Ahmed S, Kifli N, Uddin QT, Tahir NB, Hussain A, et al. Multiple pathways are responsible for Anti-inflammatory and Cardiovascular activities of Hordeum vulgare L. Journal of translational medicine. 2014; 12(1):316.

[14] Marwat S, Hashimi M, Khan K. Barley (Hordeum vulgare L.) A prophetic food mentioned in Ahadith and its ethnobotanical importance. American-Eurasian J Agric Environ Sci. 2012; 12(7):835-41.

[15] Adhikari BM, Bajracharya A, Shrestha AK. Comparison of nutritional properties of Stinging nettle (Urtica dioica) flour with wheat and barley flours. Food science & nutrition. 2016;4(1):119-24.

[16] Qasim M, Khalid M, Sayyed A, Din I, Hayat K, Jan SA. Phytochemical potentials and medicinal uses of twenty-four selected medicinal plants from Swabi, Pakistan. Journal of Rural Development and Agriculture. 2016;1(1):49-58.

[17] Min L. Research advance in chemical composion and pharmacological action of mung bean. Shanghai J Trad Chin Med. 2001; 5:18.

[18] Randhir R, Lin Y-T, Shetty K. Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. Process Biochemistry. 2004;39 (5): 637-46.

[19] Yao Y, Chen F, Wang M, Wang J, Ren G. Antidiabetic activity of Mung bean extracts in diabetic KK-Ay mice. Journal of Agricultural and Food Chemistry. 2008;56(19):8869-73.

[20] Vanamala J, Reddivari L, Yoo KS, Pike LM, Patil BS. Variation in the content of bioactive flavonoids in different brands of orange and grapefruit juices. Journal of Food Composition and Analysis. 2006; 19(2-3):157-66.

[21] Anjum NA, Umar S, Iqbal M, Khan NA. Cadmium causes oxidative stress in mung bean by affecting the antioxidant enzyme system and ascorbate-glutathione cycle metabolism. Russian Journal of Plant Physiology. 2011; 58(1):92-9.

[22] Kanatt SR, Arjun K, Sharma A. Antioxidant and antimicrobial activity of legume hulls. Food Research International. 2011; 44(10): 3182-7.

[23] Li S. Part of cereals. Ben-Cao-Gang-Mu Beijing: People's Hygiene Publishing. 1999: 1344-9.

[24] Durak A, Baraniak B, Jakubczyk A, Świeca M. Biologically active peptides obtained by enzymatic hydrolysis of Adzuki bean seeds. Food chemistry. 2013;141(3):2177-83.

[25] Livestrong. Cooking Beans and Legumes 2019 [cited 2019 6 March]. Available from: https://www.livestrong.com/article/261095-how-to-cook-yellow-split-peas/.

[26] Nadkarni KM. [Indian materia medica]; Dr. KM Nadkarni's Indian materia medica: with Ayurvedic, Unani-Tibbi, Siddha, allopathic, homeopathic, naturopathic & home remedies, appendices & indexes. 1: Popular Prakashan; 1996.

[27] Chopra R, Nayar S, Chopra I. Glossary of indian medicinal plants, publication and information directorate. CSIR New Delhi. 1992:132-210.

[28] Rubio LA, Pérez A, Ruiz R, Guzmán MÁ, Aranda-Olmedo I, Clemente A. Characterization of pea (Pisum sativum) seed protein fractions. Journal of the Science of Food and Agriculture. 2014; 94(2):280-7.

[29] Borah P. 6 Best Black Bean Recipe | Easy Black Bean Recipes: NDTV; 2019 [Available from: https://food.ndtv.com/lists/5-best-black-bean-recipes-1634986.

[30] Bennink M. Consumption of black beans and navy beans (Phaseolus vulgaris) reduced azoxymethane-induced colon cancer in rats. Nutrition and cancer. 2002;44(1):60-5.

[31] Aparicio-Fernández X, García-Gasca T, Yousef GG, Lila MA, González de Mejia E, Loarca-Pina G. Chemopreventive activity of polyphenolics from black Jamapa bean (Phaseolus vulgaris L.) on HeLa and HaCaT cells. Journal of agricultural and food chemistry. 2006; 54(6):2116-22.

[32] Takeoka GR, Dao LT, Full GH, Wong RY, Harden LA, Edwards RH, et al. Characterization of black bean (Phaseolus vulgaris L.) anthocyanins. Journal of Agricultural and Food Chemistry. 1997; 45(9):3395-400.

[33] Xu B, Chang S. Total phenolic content and antioxidant properties of eclipse black beans (Phaseolus vulgaris L.) as affected by processing methods. Journal of food science. 2008; 73(2):H19-H27.

[34] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacterio phage T4. Nature. 1970; 227(5259):680-5.

[35] He F. Laemmli-SDS-PAGE. Bio-protocol. 2011; 1(11):e80.

Citation: *Siew-Hsia Loi Julia, Lay-Harn Gam, "SDS-Page Protein Profiling of a Few Commonly Consumed Beans", Journal of Biotechnology and Bioengineering, 4(1), 2020, pp 14-19.*