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Effect of In Vitro Protein Digestibility on Bioavailable Protein in Cereals, Pseudo Cereals, Pulses and Super Seeds in both Uncooked and Cooked form

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ABSTRACT

Nutritive value of protein of any food matrix depends on protein digestibility. Protein digestibility determines bioavailability of protein. Hence it is important that one should know protein digestibility to understand nutritional value of food matrix. Purpose was to study protein digestibility and understand influence of protein digestibility on bioavailable protein. In cereals protein digestibility is ranging from 59-90%, wheat is having highest and Sorghum is having lowest. 90% protein digestibility was obtained for pseudo cereals and is comparable to wheat. Super seeds are rich in protein as well as in bioavailable protein as they exhibited superior digestibility of protein. In case of pulses all tested varieties have showed promising protein digestibility (91-84%) except for red kidney beans (75%). Also, significant difference p>0.01 was observed between protein content and bioavailable protein based on protein digestibility in both uncooked and cooked samples with few exceptions. These findings suggests that protein digestibility of food matrix is important factor.

Keywords: *In vitro protein digestibility, Cereals, Millets, Pseudo cereals, Pulses, Super seeds, Bioavailability*

1. INTRODUCTION

Food proteins have both nutritional and functional properties. Nutritional value of protein is determined by amino acid content and utilization of specific amino acids upon digestion and absorption. On the other hand, functional properties of protein is associated with physiochemical and sensory properties of foods (Joseph, Reynolds, Declan, Gerald and Catherine, 2011). Availability of amino acids in food is determined by digestibility of protein present in food source, even though nutritive value of protein is evaluated by amino acid profile (Hsu, Vavak, Satterlee and Miller, 1977). The ease of protein breakdown and the size of the products (peptides) after digestion with digestive enzymes is determining factor for bioavailability of dietary protein (Siying Wen, et al., 2015). Hence if the protein content is high and digestibility is low bioavailable protein will also be low.

In our experiment we have studied different cereals which belongs to family Poaceae like Wheat, Rice, Maize, Sorghum, Pearl millet, Kodo, Little and Foxtail millet for in vitro protein digestibility. Cereals are staple food for both developing and developed countries and important source of nutrients. For human wheat is a major source of plant protein and it is most commonly consumed cereal grain. It is a rich source of various antioxidants, minerals, vitamins and fibers. 80% of total protein content is contributed by Gluten. Millets are small seed cereals. It is consumed by large population and it is major food source. Millets are rich in calcium, protein, dietary fibre, fat and polyphenols (Issoufou, Mahamadou and Guo-Wei Le., 2013). Pseudocereals are non-grass species either fruits or seeds. Its nutritive value is comparable to conventional crops and consumption is similar to cereals. With respect to protein content and protein quality pseudocereals are better than cereals. Unlike cereals, pseudocereals lack enzyme inhibitors and allergens (Saubhik Das, 2016). In our experiment we have studied two pseudocereals Quinoa and Buckwheat. In many parts of the world nuts and seeds have contributed nutrition significantly to human population. Usually these are consumed together with other food items or also consumed as snacks (H. Glew, S. Glew, Chuang, Huang and Millson, 2006). We have analyzed 3 super seeds Sunflower seed, Chia seed and Pumpkin seed. Sunflower seeds are oil seeds and these protein rich seeds are one of the most cultivated oil crops (Liu, Li, Zeng, Xu, Wang, Zhang, and Piao, 2015). Chia seeds are rich source of protein, fat, dietary fibre and polyphenols. As compared to cereals, protein content of chia seeds is high. It can be digested by patients suffering from celiac disease because of absence of gluten (Rahman, Nadeem, Khalique, Imran, Mehmood, Javid and

Hussain, 2016). Like other seeds pumpkin seeds have high level of crude oil and crude protein. Seed kernel of pumpkin seed possess dietary and medicinal values (Esam Erno, Andrea, Eva and Anna, 1992). Protein content in pulses are twice of protein content present in cereals. Protein content of meal is enhanced when pulse and cereal are eaten in combination (Ofuya, Akhidue, 2005). In our study we have covered 6 varieties of pulses belonging to family Fabaceae including lentil, bean and pea. Lentils apart from being rich in protein they are associated with lowering cholesterol and lipid in humans. Lentils have high phenolic, flavonoid and condensed tannins (Marcela, 2017). In beans, red kidney beans are good source of vegetable protein, starch, soluble and insoluble fiber, vitamins (especially B group) and minerals (Ibeabuchi, Peter; Agunwa, Eluchie, Ofoedu and Nwatu, 2017).

In this study our hypothesis is, due to protein digestibility there is a significant difference between protein content and bioavailable protein. To prove this hypothesis, we have covered 8 varieties of cereals, 2 varieties of pseudo cereals, 3 varieties of super seeds and 6 varieties of pulses. Protein digestibility for all these food matrices were analyzed and on the basis of protein digestibility value bioavailable protein was calculated.

2. MATERIAL AND METHOD

2.1. Samples

Flour of Wheat, Maize, Kodo millet, Little millet Foxtail millet, Buckwheat and Soybean were procured from super market in Bangalore, India with particle size ranging from 100-250 micron. These samples were directly used for experiments. Rice, Sorghum, Pearl millet, Quinoa, Red Lentil, Split pigeon pea, Split mung beans, Red kidney beans, Brown chickpea, Chia seeds, Sunflower seeds and pumpkin seeds were procured from super market in Bangalore, India as whole grain and seed. These whole grain and seed samples were ground to powder with particle size ranging from 100 - 600 micron, using mixer and then used for experiments. Cooked samples; Wheat, Rice, Maize, Pearl millet, Sorghum, Buckwheat and soybean flour are made into a dough using water. This dough is rolled on flat surface to make a thin layer followed by cooking it on a hot pan for approximately 5 minutes/until it is cooked. Kodo millet, Little millet, Foxtail millet and Quinoa are boiled in water till it is cooked. Split mung bean, Split pigeon pea, Red lentil, brown chickpea and Red kidney beans are cooked using pressure cooker.

2.2 Chemicals

Pepsin and Pancreatin enzymes were procured from Sigma Aldrich. Sodium hydroxide (NaOH) pellets, Hydrochloric acid (HCl), Trichloroacetic acid (TCA) $C_2HC_{l3}O_2$, Boric acid (H_3BO_3), Copper sulphate Pentahydrate ($CuSO_4.5H_2O$), Sulphuric acid (H_2SO_4), Sodium di hydrogen phosphate (NaH_2PO_4), Disodium hydrogen phosphate (Na_2HPO_4) and sodium sulphate anhydrous (Na_2SO_4) were procured from Merck. Toluene (C_7H_{18}) was procured from S.D. Fine chemicals.

2.3 In vitro protein digestibility

In vitro protein digestibility was determined according to the method of Akeson and Stahmann, (1964). One gram sample treated with 15 ml of Pepsin enzyme (1.5 mg) dissolved in 0.1 M HCl. Sample was incubated with enzyme at 37°C under shaking condition, 150rpm for 3 hours in shaking water bath. After completion of 3 hours suspension was neutralized with 7.5 ml of 0.2M NaOH. This step was followed by treatment of suspension with 7.5 ml of Pancreatin enzyme (4 mg) prepared in 0.2 M phosphate buffer (pH 8.0). Toluene (1ml) was added to prevent microbial growth. Mixture was incubated for additional 24 hours at 37°C under shaking condition. After completion of 24 hours, sample was treated with 10 ml of 10% TCA to remove undigested protein and larger peptides and kept at 2-8 ºC for 30mins. After this mixture was centrifuged at 10000 rpm for 15 min at room temperature. All the samples were studied in duplicates and 3 independent experiments were carried out. Protein content of sample (as such) and pellet obtained after in vitro digestion was estimated by Gerhardt Vapodest 50S method (IS: 7219-1973). Percentage of protein digestibility was calculated by the ratio of protein in supernatant to protein in sample as equation:

$$\%\ \text{Protein digestibility} = \frac{(\text{Total Nitrogen in Sample} - \text{Total Nitrogen in pellet})}{\text{Total Nitrogen in sample}} \times 100$$

Bioavailability of protein for the sample was calculated using Protein content of sample and % in vitro protein digestibility of the sample.

$$\text{Bioavailable protein} = \text{Protein content} \times \%\ \text{Protein digestibility}$$

2.4 Statistical analysis

Data are presented as mean ± standard deviation (n=3). To determine the statistical differences analysis of variance (ANOVA) was performed. Two-way ANOVA was utilized for evaluating the differences. $p < 0.05$ and $p < 0.01$ were considered as significant.

3. RESULT AND DISCUSSION

In vitro protein digestibility was studied using pepsin – pancreatin enzyme method. Cereals, Millets, Pseudo cereals and Pulses were analyzed in both uncooked and cooked form and super seeds were analyzed in uncooked form using this method.

3.1 Cereals and Millets

Eight different cereals and millets were tested in both uncooked and cooked form to understand protein digestibility. Protein digestibility of Wheat is significantly high as compared to other cereals and millets. The value obtained is in agreement with value reported by Nils B. Buchmanna, (1977) i.e.88%. Since in wheat, digestibility of protein is high (91%) bioavailable protein is also high. To elaborate it further, 11.8 gm of protein is bioavailable for an individual if consumed 100 gm of wheat or 1.18 gm is bioavailable if consumed 10gm. Rice and Maize are having protein digestibility (Figure 1) 80% and 83% respectively. This data indicates that though the protein content of rice is high, protein digestibility is lower than maize. As discussed in research article by A. Bandegan et al, 2010 and V Ravindran, Hew, G. Ravindran and Bryden (1999), digestibility of individual amino acids plays crucial role. In wheat; Alanine, Aspartic acid, Lysine, Threonine and Glycine are present in lower proportion as compared to rest and these amino acids are having lower digestibility. All these amino acids (refer table 1) are high in rice (Ejeta, Hassen, And Mertz, 1987) as compared to wheat (Jiang, Tian, Ha Zhi and Zhang, 2008) hence may be protein digestibility of rice is less as compared to wheat. Similarly, in maize (Ejeta, Hassen, And Mertz, 1987) these amino acids are high with respect to wheat, hence this could be the reason for low protein digestibility as compared to wheat. Bioavailable protein (refer Table 3) for wheat is high as compared to rice and maize. In millets, Protein digestibility is approximately 29- 19% lesser than wheat. This may be attributed to high amount of Aspartic acid, Alanine and Threonine in pearl millet, Foxtail millet (Issoufou, Mahamadou and Guo-Wei Le., 2013), Kodo millet and little millet (Pasala and Bjorn, 1989) and also lower concentration of amino acids like Proline and arginine with respect to wheat. Proline and Arginine is having high digestibility as discussed by Bandegan et al, 2010. Sorghum is having lowest digestibility as compared to rest of cereals and millets. As mentioned in book Sorghum and Millets: Chemistry, Technology, and Nutritional Attributes (2018) by John and Duodu, like other cereals, pericarp of Sorghum is rich in cellulose, but unlike other cereals (endosperm is rich in soluble arabinoxylans or β-glucans) endosperm of sorghum is rich in water unextractable glucorunoarabinoxylans. Because of this insoluble dietary fibre in sorghum ranges from 94-96%, whereas for wheat it is 85%. Similarly, high value of insoluble dietary fibre is present in pearl millet ranging from 89-97%. Hence this could be one of the reasons for low protein digestibility of Sorghum and Pearl millet with respect to Wheat. Also as discussed in review article by Duodu, Taylor, Belton and Hamaker, 2003 there are endogenous and exogenous factors which can affect protein digestibility of Sorghum. Exogenous factors like grain organizational structure and endogenous factors like interaction of polyphenols, phytate, non-starch polysaccharides and starch which results in low protein digestibility. In our study protein digestibility of pearl millet is significantly high as compared to Sorghum, Kodo Millet, Little millet and Foxtail millet. In vitro protein digestibility value for pearl millet is 72% and Sorghum it is 59 %, (Figure1, Table 3) however protein content for both the cereals are same. On the other hand, protein content of foxtail millet is 14% and in vitro protein digestibility is 61%. Bioavailable protein for Foxtail millet is higher than Sorghum and other tested millets, because of high protein content. As discussed Protein content of wheat is 11% and Foxtail millet is 14%. Though protein content is high in Foxtail millet, bioavailable protein is less as compared to wheat, as protein digestibility (Figure1 and Table 3) for foxtail millet is 60.6% and 89.5% for wheat. Cereals like Wheat, Kodo millet, little millet and Foxtail millet did not show significant difference in total protein in uncooked and cooked form. However, Kodo millet and little millet showed significant difference in bioavailable protein. Rice, Maize, Pearl millet and Sorghum showed significant difference in both total protein as well as bioavailable in uncooked and cooked condition. When compared % Protein digestibility in uncooked and cooked form except for Maize and Little millet rest all have shown no significant difference (Fig 1).

3.2 Pseudo Cereals

Pseudo cereals are also good source of protein. We have studied two pseudo cereals Quinoa and Buckwheat. In vitro protein digestibility (Figure 2, Table 3) values for Quinoa and Buckwheat are approximately similar with difference of 1% as per Pepsin Pancreatin method. Unlike grains, pseudo cereals like quinoa and buckwheat proteins are composed mainly of globulins and albumins. These pseudo cereals contain very low prolamin proteins, which are storage proteins found in cereals (Oksana, Mária, Jana, Mária, Matúš and Marián 2018). Also as discussed in review article by Christa K. and Maria (2008) with comparison to wheat, barley and rye, Buckwheat prolamins are differently characterized, hence buckwheat can be consumed by population having prophylactic of gastrointestinal tract disease like Celiac disease. Amino acid profile for Buckwheat (Yeshajahu and George, 1972) and Quinoa (Olga Escuredo, et al, 2014) are listed in Table 1. The amino acid composition of globulins and albumins differs significantly from that of prolamins, which has implications in relation to their nutritional quality. May be because of this difference in protein content pseudocereals are having high protein digestibility as compared to cereals and millets except for Wheat. Bioavailable protein for Buckwheat is less as protein content of Buckwheat is 8.4% and for Quinoa it is 14.6% (Table 3). Both quinoa and buckwheat did not show significant difference in total protein and bioavailable protein in uncooked and cooked form. However, uncooked buckwheat showed significant difference in % Protein digestibility as compared to cooked buckwheat (Fig 1).

Table 1: Amino acid profile for cereals, millets and pseudo cereals. Analysis was not carried out, values are referred from research articles (mentioned in brackets). Values represented in g/100g.

g/100g	Cereals and Millets								Pseudo cereals	
Amino acids	Rice (Ejeta, Hassen, And Mertz, 1987)	Wheat (Jiang, Tian, Ha Zhi and Zhan, 2008)	Maize (Ejeta, Hassen, And Mertz, 1987)	Sorghum (Ejeta, Hassen, And Mertz, 1987)	Pearl millet (Issoufou, Mahamadou and Guo-Wei Le., 2013)	Kodo millet (Pasala and Bjorn, 1989)	Little millet (Pasala and Bjorn, 1989)	Foxtail millet (Issoufou, Mahamadou and Guo-Wei Le., 2013)	Quinoa (Yeshajahu and George, 1972)	Buck wheat (Olga Escuredo, et al, 2014)
Alanine	9.8	5.8	7.75	8.85	8.1	8.88	9.61	9.3	2.19	4.5
Arginine	6.1	8.6	4.4	4	0.9	3.34	3.14	3	3.08	9.7
Aspartic acid	7.9	3.8	7.55	7.1	6.2	5.59	5.35	7.71	4.24	11.3
Cystine	1.4	2.5	2.1	1.45	0.8	1.52	1.46	0.45	0.12	1.6

Glutamic acid	19.1	25.4	20.8	21	22.8	20.39	22.34	22	8.79	18.6
Glycine	5	3.6	3.8	3.35	0.7	2.27	1.81	2.91	3.19	6.3
Histidine	2.7	2.1	3.2	2.25	1.7	1.89	2.01	2.11	1.89	2.7
Isoleucine	4.1	4.4	3.8	3.85	5.1	3.57	4.91	4.59	0.79	3.8
Leucine	8.9	13.2	13.4	13.1	14.1	8.98	11.41	13.6	2.39	6.4
Lysine	4	2.1	2.85	2.5	0.5	1.33	1.09	1.59	2.36	6.1
Methionine	3.2	1.7	2.15	1.7	1	3.33	3.07	3.06	0.46	2.5
Phenylalanine	5.5	4.7	5.2	5.15	7.6	7.84	5.94	6.27	1.5	4.8
Proline	4.8	10.6	10.05	8.1	8.2	7.23	6.89	5.54	1.81	3.9
Serine	5.4	4.8	5.1	4.4	5.4	4.94	6.39	4.56	1.7	4.7
Threonine	3.9	2.1	3.85	3.3	3.3	3.56	3.55	3.68	6.47	3.9
Tryptophan	-	0.9	0.8	0.9	1.2	1.5	0.69	NA	1.02	-
Tyrosine	3.7	3.0	4.3	3.85	2.7	5.74	4.37	2.44	1.15	2.1
Valine	6.5	6.1	5.05	5.1	4.2	5.01	6.23	5.81	0.9	5.1

Table 2: Amino acid profile for super seeds and Pulses. Analysis was not carried out, values are referred from research articles (mentioned in brackets). Values represented in g/100g.

g/100g	Super seeds			Pulses					
Amino acids	Chia seed (Soňa, Matej, Jaroslav, Peter, Rafay and Alexander, 2014)	Sun flower seed (Soňa, Matej, Jaroslav, Peter, Rafay and Alexander, 2014)	Pumpkin seed (Soňa, Matej, Jaroslav, Peter, Rafay and Alexander, 2014)	Split mung bean (Chan, Ling and Ching, 2009)	Split Pigeon pea (Matthew G. Noswort hy,Jason Neufeld et al, 2017)	Red Lentil (Nazma, Saiful, Sarah, Md. Mohiduzzaman and Thingnganing, 2016)	Brown chick pea (Paredes, Ordorica, and Olivares, 1991)	Red Kidney bean (Matthew G. Noswort hya et al., 2017)	Soy Bean (Van Etten, Hubbard, Jean, Smith, and Blessin, 1959)
Alanine	0.9	0.95	1.32	4.01	1.09	1.43	5	1.15	4.03
Arginine	2.0	2.04	4.59	8.26	1.93	0.84	11.1	1.43	6.46
Aspartic acid	1.3	2.05	2.56	12.18	2.86	0.63	11.7	3	11.3
Cystine	0.4	0.11	0.1	0.21	0.31	1.05	2	0.22	1.36
Glutamic acid	2.9	4.37	5.19	19.64	4.08	2.02	18.3	3.91	18.5
Glycine	0.9	1.11	1.29	3.28	1.08	2.12	3	0.88	4.6
Histidine	0.6	0.58	0.76	3.71	0.61	0.22	2.1	0.9	2.59
Isoleucine	0.7	0.92	1.15	37.9	0.98	0.15	4.3	0.96	5.26
Leucine	1.4	1.44	2.16	7.71	1.84	1.03	8	2	8.13
Lysine	0.9	0.82	1.14	7.22	1.82	0.26	6.1	1.46	6.67
Methionine	0.7	0.47	0.65	1.24	0.26	1.37	1.5	0.24	
Phenylalanine	1.2	1.06	1.54	6.07	1.19	2.27	3.7	1.31	5.61
Proline	1.3	0.74	0.92	4.39	1.04	1.92	1.9	0.65	5.32
Serine	0.9	0.82	1.19	6.38	1.25	3.23	5.5	1.55	5.97
Threonine	0.5	0.79	0.71	4.11	0.96	5.48	3.4	0.99	3.93
Tryptophan	-	0.34	0.56		0.2	1.15		0.26	1.35
Tyrosine	0.6	0.67	1.09	3.3	0.73	1.08	2.8	0.66	4.37
Valine	0.8	1.12	1.49	4.65	1.1	1.52	4.3	1.19	5.57

3.3 Super Seeds

In our experiments we have also studied super seeds like Sunflower seed, Chia seed, Pumpkin seed (both Green and White). Amino acid profile for Chia seed (Soňa, Matej, Jaroslav, Peter, Rafay and Alexander, 2014), Sunflower seed and Pumpkin seed (R. G. Robinson, 1975) are listed in Table 2. It was observed that Alanine, Glycine, Isoleucine, Leucine, lysine, Serine and Tryptophan are high in concentration in pumpkin seed as compared to Chia and Sunflower seed. As discussed in research article by Liu, Li, Zeng, Xu, Wang, Zhang, and Piao (2015) and V Ravindran, Hew, G. Ravindran and Bryden (1999), wherein they have studied illeal digestibility of amino acids in sunflower seed meal, all these amino acids are having low digestibility (below 75%). Therefore, this may be the reason for low protein digestibility (refer Figure 2, Table 3) of pumpkin seed (80%) as compared to chia seed (94%) and sunflower seed (95%). Both Green and white variety of pumpkin seeds showed similar protein digestibility values although slight difference in protein content was observed 35% and 33% respectively. Since % protein digestibility of pumpkin seed is less, % Bioavailable protein for pumpkin seeds are approximately similar to sunflower seed (protein content 29%) in spite of having high protein content.

Table 3: Total protein, % *In vitro* protein digestibility and % Bioavailable protein values for all tested samples in uncooked form. Data represents values of three independent experiments, N=3, Mean ± SD. ** p value <0.01, * p value<0.05, Significant difference between Protein content and Bioavailable protein.

Food Product Uncooked	Protein (%)	Protein Digestibility (%)	Bioavailable Protein (%)
Cereals and Millets			
Wheat	12.7 ± 0.12	91.0 ± 3.3	11.6 ± 0.34**
Rice	11.7 ± 0.1	80.1 ± 1.1	9.3 ± 0.14**
Maize	7.64 ± 0.15	83.2 ± 2.6	6.4 ± 0.32**
Sorghum (Jowar)	11.8 ± 0.12	64.6 ± 3.6	7.6 ± 0.5**
Pearl millet (Bajra)	11.2 ± 0.22	75.6 ± 5.1	8.5 ± 0.65**
Kodo millet	9.3 ± 0.07	68.9 ± 0.9	6.41 ± 0.13**
Little millet	11.04 ± 0.19	77.1 ± 1.6	8.5 ± 0.05**
Foxtail millet (Italian millet)	16.3 ± 0.19	66.1 ± 1.61	10.8 ± 0.24**
Pseudo cereals			
Quinoa	15.0 ± 0.26	89.9 ± 3.0	13.5 ± 0.59*
Buckwheat	9.1 ± 1.6	90.0 ± 2.0	8.2 ± 1.4
Super seeds			
Sunflower seed	30.6 ± 0.24	95.5 ± 1.1	29.2 ± 0.47*
Chia seed	25.6 ± 1.05	94.8 ± 0.5	24.2 ± 1.0
White Pumpkin seed	39.5 ± 0.81	82.3 ± 1.7	32.5 ± 0.7**
Green Pumpkin seed	36.7 ± 1.04	82.9 ± 4.3	30.2 ± 2.0*
Pulses			
Split mung beans (Moong dal)	30.8 ± 0.45	86.7 ±1.2	26.7 ± 0.73**
Split pigeon peas (Toor dal)	27.3 ± 3.00	89.5 ± 2.3	24.5 ± 3.3
Red Lentil (Masoor dal)	30.0 ± 2.3	92.1 ± 1.0	27.5 ± 2.4
Brown chickpea (Kala chana, Desi chickpea)	22.0 ± 0.26	92.8 ± 0.3	20.4 ± 0.18**
Red Kidney bean (Rajhma)	21.5 ± 0.0.55	79.4 ± 1.9	17 ± 0.36**
Soybean	64.1 ± 0.51	89.9 ± 1.1	57.6 ± 0.55**

Table 4: Total protein, % *In vitro* protein digestibility and % Bioavailable protein values for all tested samples in cooked form. Data represents values of three independent experiments, N=3, Mean ± SD. ** p <0.01, * p <0.05, Significant difference between Protein content and Bioavailable protein.

Food Product Cooked	Protein (%)	Protein Digestibility (%)	Bioavailable Protein (%)
Cereals and Millets			
Wheat	11.2 ± 1.2	89.3 ± 2.5	10.0 ± 1.34
Rice	9.7 ± 0.9	77.0 ± 8.3	7.5 ± 1.5
Maize	7.3 ± 0.04	71.9 ± 4.0	5.24 ± 0.3**
Sorghum (Jowar)	9.6 ± 0.73	60.6 ± 7.6	5.8 ± 0.61*
Pearl millet (Bajra)	8.7 ± 1.12	68.8 ± 2.8	6.04 ± 1.0**
Kodo millet	9.2 ± 0.16	64.9 ± 3.6	6.0 ± 0.24**
Little millet	11.0 ± 0.35	63.5 ± 4.3	7.0 ± 0.44**
Foxtail millet (Italian millet)	15.7 ± 1.5	68.8 ± 2.1	10.8 ± 0.73**
Pseudo cereals			
Quinoa	14.2 ± 2.75	89.0 ± 1.7	12.7 ± 2.67
Buckwheat	7.4 ± 0.56	82.8 ± 2.6	6.12 ± 0.41*
Pulses			
Split mung beans (Moong dal)	29.8 ± 1.4	85.1 ± 2.16	25.3 ± 1.8*
Split pigeon peas (Toor dal)	26 ± 0.52	82.0 ± 3.76	21.3 ± 1.3**
Red Lentil (Masoor dal)	29.3 ± 0.42	90.3 ± 0.14	26.5 ± 0.4**
Brown chickpea (Kala chana, Desi chickpea)	22.2 ± 1.6	91.0 ± 2.6	20.2 ± 1.9
Red Kidney bean (Rajhma)	17.6 ± 2.0	73.0 ± 8.22	13.0 ± 2.3*
Soybean	62.7 ± 1.21	87.1 ± 2.0	54.6 ± 0.23**

3.4 Pulses

We have studied 6 pulses including Red lentil (Masoor dal), Split pigeon pea (Toor dal), Brown chickpea (Bengal gram/ Desi chickpea/ Kala chana), Split mung bean (mung dal), Red kidney bean (Rajhma) and Soy bean. Lentils as discussed in review article by Marcela Jarpa-Parra (2017), are rich in protein. These proteins are storage proteins classified as albumins, globulins and glutelins. Lentils have high dietary fibre and they are rich in soluble fibre than bean and chickpeas. Amino acid profile (Nazma, Saiful, Sarah, Md. Mohiduzzaman and Thingnganing, 2016) for red lentil is listed in Table 2. Protein digestibility of red lentil as reported by Matthew G. Nosworthya et al., (2017), was 88.01 and Matthew, Jason, Peter, Gina, Linda and James (2017) was 90.6% which is in agreement with our study, 90.13% (refer Figure3, Table3). Amino acid profile of split pigeon pea (Matthew G. Nosworthy,Jason Neufeld et al, 2017) and brown chick pea (Paredes, Ordorica, and Olivares, 1991) are listed in Table2. In case of peas, Split pigeon pea is having high protein content as compared to brown chick pea. Difference in bioavailable protein is approximately 4% for split pigeon pea and brown chickpea, whereas difference in protein content is 5% (refer Figure3, Table 3). This is because of high protein digestibility of brown chickpea when compared with split pigeon pea. Low digestibility of split pigeon pea may be because of antinutritional factors as discussed in research article by Matthew G. Nosworthy, Jason Neufeld (2017) like proteolytic inhibitors and tannins. In case of beans as represented in Table 3, Soybean is having highest protein content (52%) followed by split mung bean (25.6%) and Red kidney bean (19.7%). The trend is same for % in vitro protein digestibility, Soybean 88.7%, Split mung bean 84% and Red kidney bean 75.2%. Low protein digestibility of Red kidney beans may be because of different antinutrients, Phytic acids, polyphenols and condensed tannins. These components form complexes with protein thereby increasing crosslinking and decreasing solubility of protein. This results in low susceptibility of protein complex to proteolytic enzymes as discussed by Alonso, Aguirre, Marzo 1999. Amino acid profile for Soybean (Van Etten, Hubbard, Jean, Smith, and Blessin, 1959), Split mung bean (Chan, Ling and Ching, 2009) and Red kidney bean (Matthew G. Nosworthy et al., 2017) are listed in Table 2. In case of uncooked and cooked form all tested pulses and beans did not show significant difference in total protein and bioavailable protein, exceptions were Red kidney bean and Soybean which showed significant difference in total protein and bioavailable protein respectively. Split pigeon pea and Red lentil showed significant difference in % Protein digestibility, rest all tested samples did not show.

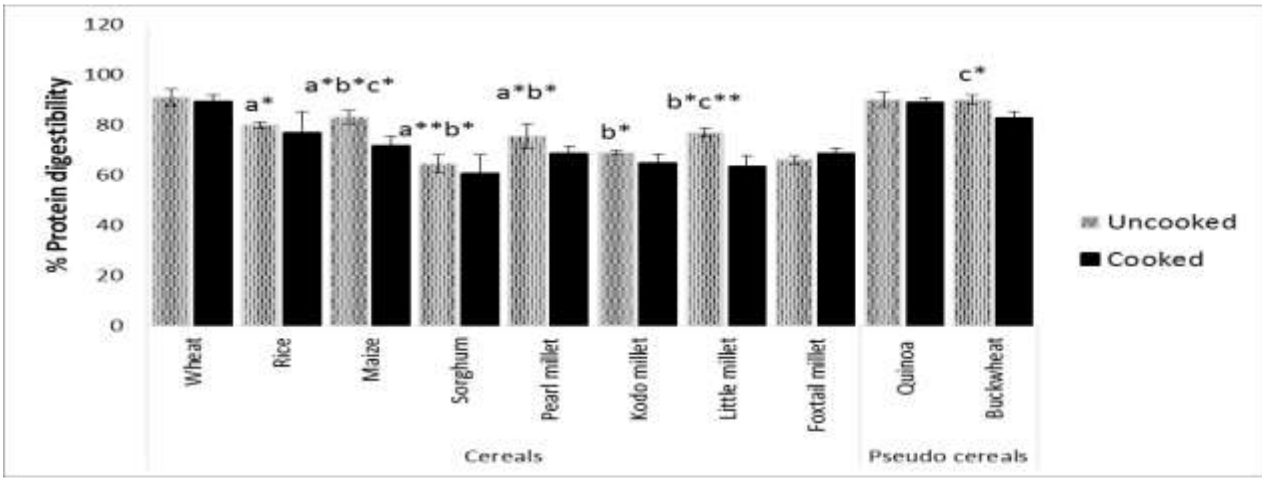

Figure 1: Percentage *In vitro* protein digestibility of various cereals. Values are mean of three independent experiments. Standard deviations are indicated. N=3, Mean ± SD. In the graph 'a' indicates significant difference in total protein, 'b' indicates significant difference in bioavailable protein and 'c' indicates significant difference in protein digestibility between uncooked and cooked samples, $p<0.05$*, $p<0.01$**

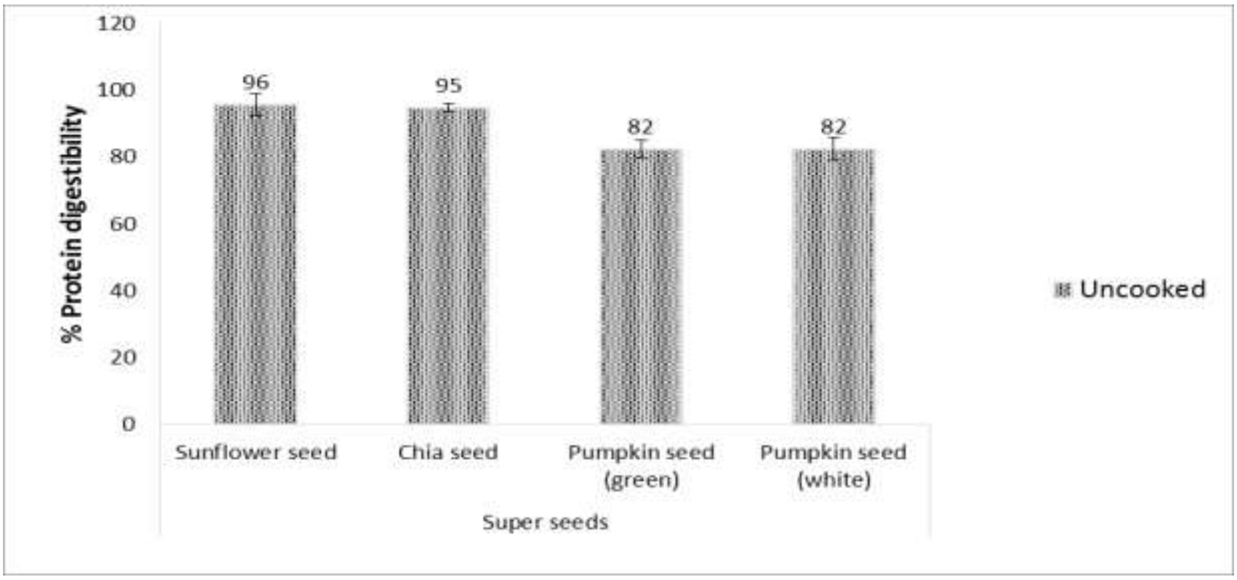

Figure 2: Percentage In vitro protein digestibility of various Pseudo cereals and super seeds. Values are mean of three independent experiments. Standard deviations are indicated. N=3, Mean ± SD

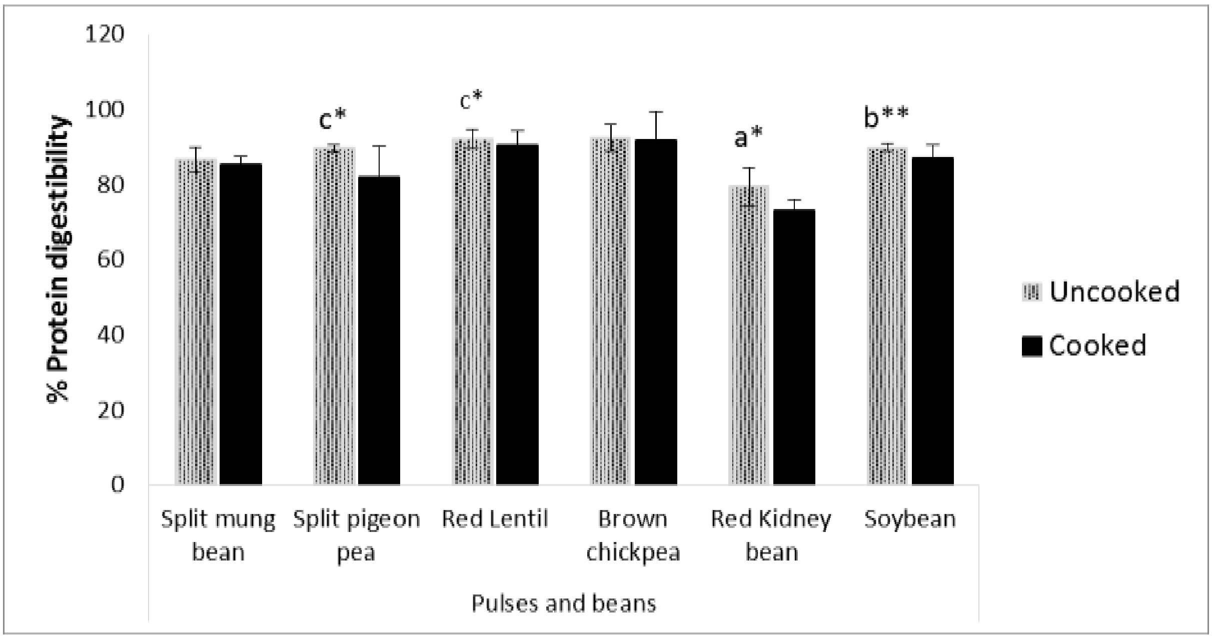

Figure 3: Percentage *In vitro* protein digestibility of pulses, lentils, peas and beans. Values are mean of three independent experiments. Standard deviations are indicated. N=3, Mean ± SD. In the graph 'a' indicates significant difference in total protein, 'b' indicates significant difference in bioavailable protein and 'c' indicates significant difference in protein digestibility between uncooked and cooked samples, p<0.05*, p<0.01**

4. CONCLUSION

In our experiment we have studied nineteen samples for In vitro protein digestibility using Pepsin pancreatin protein digestion method. The study was mainly carried out to understand the protein digestibility and bioavailable protein for cereals, millets, pseudo cereals, super seeds and pulses. Our hypothesis, hence proved showing significant difference between protein content and bioavailable protein based on protein digestibility of sample. In uncooked form, all the samples showed significant difference, except for Buckwheat, Chia seeds, Split pigeon pea and red lentils. In case of cooked form except for Wheat, Rice, Quinoa and Black chickpea, rest all samples have shown significant difference between protein content and bioavailable protein. In case of foxtail millet, Sorghum and Red kidney bean though protein content is high, bioavailable protein has reduced by approximately 5%, because of low protein digestibility. In contrast, wheat and buckwheat protein content is less but most of the consumed proteins are bioavailable since digestibility of protein for these food material is high. Hence based on protein content one cannot decide the nutritional value for food matrix. Because if protein digestibility is low, it will reduce bioavailable protein to human body. If bioavailability of nutrients is one major aspect, absorption of bioavailable nutrients is another major factor. In this study we have not addressed bio absorption of protein/ peptides. It can be studied further to understand bio absorption of bioavailable proteins.

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