Biofortification of pulses and legumes to enhance nutrition

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1. Introduction

Malnutrition is a grave concern and is widely spread in most African and Asian nations. It has gained the world attention due to its severe associated health problems. Each year twenty million infants are born with low birth weight. As per the WHO (2018) malnutrition reports, 150.8 million, 50.5 million, and 38.3 million of children under 5 years of age are found to be stunted, wasted and overweight respectively whereas 38.9% of adults are found to be overweight or obese from Africa to North America [1]. One third of all Women of reproductive age are anemic, thus women are more vulnerable to certain forms of malnutrition and also they have a higher prevalence of obesity as compare to men [1]. Approximately 38.1% of women of reproductive age have anemia, 8.1% have diabetes and 17% have obesity in Africa [43]. Malnutrition generally affects all the classes in a society, but the infants, babies and pregnant women are most vulnerable. Globally various policies have been implemented to address the problem of malnutrition such as National Nutrition Mission and National Food Security Mission in India [44]. But due to some economic reasons and natural calamities, malnutrition is still a most significant challenge for food scientists and policy makers. Also, in the recent years due to shift in the eating habits by changes in lifestyle, urbanization and globalization, people started consuming junk and ready to eat foods on a larger scale which generally have high content of sugar, fat, salt and have low nutrient value in terms of protein, fiber, vitamins and mineral content. Consumption of these nutrient-deficient foods eventually leads to malnutrition and associated syndromes [2,3]. Although, a number of foods have been fortiﬁed and enriched to overcome this issue, but the problem associated with the fortiﬁed foods that they are expensive and cannot be afforded by the low-income group. So, the best solution to overcome this problem seems to be the consumption of naturally balanced diet for which the pulses and legumes are very vital and the addition of legumes/pulses to the ready to eat/cook foods like biscuits, noodles, soups, snacks, etc. in order to enhance their nutritional as well as phytochemical content.

2. Pulses and legumes

The word “pulse” is derived from a Latin word “puls or multis” meaning thick slurry. Pulses belong to the Legume family, which has been consumed for thousands of years as a part of traditional diet throughout the world. Every pulse is a legume but not vice versa like dry peas, lentils, chickpeas, dry beans, etc are pulses whereas legumes include soybeans, peanuts, fresh peas etc [37]. Legume can be deﬁned as “pod or fruit” containing seeds or dry grains and has the tendency of being nitrosonic in soil. According to FAO stat, 2018 total world production of pulses is more than 92 million tonnes in which only Asia accounts for more than 42 million tonnes [66]. India being the largest producer of the pulses (26% of global production), is also the largest consumer (27% of world consumption) and its importer (14%) in the world [67]. In last five years India has seen a substantial growth in area...
under pulse cultivation from 26 million ha in 2013 to 36.5 million ha in 2018 [66].

More than 1000 of legumes are known to be grown worldwide but only 20 of them are cultivated for consumption [65] such as chickpea (Cicer arietinum L.), pigeon pea (Cajanus cajan L.), cowpea (Vigna ungui-culata), mung bean (Vigna radiata L.), urd bean (Vigna mungo L.), lentil (Lens culinaris Medik), French bean (Phaseolus vulgaris), horse gram (Macrotyloma uniflorum), field pea (Pisum sativum L.), moth bean (Vigna aconitifolia), soybeans (Glycine max), lathyrus (Lathyrus sativus L.), etc. are grown. Table 1 contains principle constituents of some major legume seeds. Globally there is growing trend in consumption of pulses in view of their high protein content, low in calories and glycemic index. Therefore pulse in combination with other cereals provide immense opportunity to be used as an important ingredient in food processing and developing new food products such as soups, cereal bars and have various health benefits which has been discussed in detail in later part of this paper. Thus there are many types of pulses which constitute variety of nutrients and have various health benefits but this paper will discuss only soybean, mungbean and peas in detail.

3. Nutritional value of soybean, mungbeans and peas

3.1. Soybean

Soybean (Glycine max) is one of the important crop consumed globally, with the production of more than 330 million tons in 2016. United States with annual production of 117 million tons of soybean in 2016 is the largest producer followed by Brazil (53.4 million tons), Argentina (34.6 million tons) China (14.4 million tons) and India (8.6 million tons) [4].

3.1.1. Protein

Protein content of Soybean is about (37–42%) [5]. In soybean, large number of the proteins exist as storage proteins which is globulin, and the major components of globulin are β-conglycinin (7S) and glycinin (11S) [70,71]. Soy protein has low sulfur-containing amino acids, but it contains all the amino acids essential for functioning of human body namely isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan, valine, and histidine [5].

3.1.2. Lipids and carbohydrates

It contains substantial amount of oil, about 17–19% of soybean dry weight. Soybean oil is full of unsaturated fatty acids, linoleic acid, and linolenic acid and hence suitable for human consumption [5]. The composition of fatty acid in soybean oil has been estimated as palmitic acid (4–23%); stearic acid (3–30%); oleic acid (25–86%); linoleic acid (25–60%); and linolenic acid (1–15%). Soybean contains approx 35% of carbohydrate, and the majority of them are fiber. Raffinose and stachyose are the major soluble carbohydrate which accounts for 0.1%–0.9% and 1.4%–4.1% on a dry matter basis respectively [6]. Cellulose, Hemicellulose, pectin, and starch are the principal insoluble carbohydrate found in soybean.

3.1.3. Vitamins

Soybean is a good source of both water-soluble as well as fat-soluble vitamins. Thiamin, riboflavin, niacin, pantothenic acid, and folic acid are the water-soluble vitamins whereas four fat-soluble vitamins A, D, E and K are also present in soybean. The amount of Vitamin E (Tocopherol) present in soybean largely depends upon its variety. The amount of α- and δ-tocopherol in soybean ranges from 10.9 to 28.4, 150 to 191, and 24.6–72.5 μg/g (on a dry matter basis), respectively [7].

3.1.4. Minerals

It contains 5% ash. Major minerals present in soybean are potassium, sulfur, phosphorus, magnesium, calcium, chloride, and sodium found in the range of 0.2–2.1% whereas the silicon, iron, zinc, magnesium, copper, cobalt, cadmium, lead, arsenic, chromium are present in the range of 0.01–140 ppm [8,9].

3.1.5. Bioactive compounds

Daidzein, genistein, and glycitein are the three basic type of isoflavones present in the soybean (0.1–0.4% dry weight), which are not found in most of the food products [45,46]. In soybean there are twelve isomers of isoflavones and among these major ones are 6′-O-malonylgenistin, genistin, 6′-O-malonyldaidzin, and daidzein. Many factors such as variety, growing location, year and date of planting, as well as date of harvesting influence content & distribution of isomers in soybeans. For example, in a study at eight different locations it was observed that, Vinton 81, a simple variety of soybean contain isoflavone in the range of 0.84–1.64 mg/g raw seeds in year 1995 while it ranges from 1.61 to 2.84 mg/g in year 1996 [72]. Similarly in one more study it was found that the isoflavone concentration of a single variety soybean in different plantation year at different location, have up to a 5 fold differences [46].

Soy proteins contain 0.1–0.3% saponin [10]. There are three groups of soya saponin termed as group A, group B, and group E. In a study it was found that saponin composition in soybean depends upon the variety and degree of maturity rather than year of cultivation [73]. Saponin is considered as an antinutritional factor, but recent studies shows that saponins have blood cholesterol-lowering properties. The phytosterol content is in the range of 0.3–0.6 mg/g, and the three significant phytosterols present in soybean are Campesterol, β-sitosterol, and stigmasterol. The Range of phytyte content varies from 1.00 to 1.47% on a dry matter basis, and this value represented 51.4–57.1% of the total phos- phorus in seeds [11]. Kunitz inhibitor and Bowman-Birk inhibitor are the two types of trypsin inhibitors found in soybean which adversely affect the growth and causes pancreatic hypertrophy.

### Table 1. Major constituents (% of seeds weight) of legume seeds [69].

| Legume         | % Protein | % Oil     | % Starch | % Fibre | % Sucrose |
|----------------|-----------|-----------|----------|---------|-----------|
| Soybean        | 35.1–42   | 17.7–21.0 | 1.5      | 20      | 6.2       |
| Common bean    | 20.9–27.8 | 0.9–2.4   | 41.5     | 10      | 5         |
| Pea            | 18.3–31   | 0.6–5.5   | 45       | 12      | 2.1       |
| Faba bean      | 26.1–38   | 1.1–2.5   | 37–45.6  | 7.5–13.1| 0.4–2.3   |
| Lentil         | 23–32     | 0.8–2     | 46       | 12      | 2.9       |
| Chick pea      | 15.5–28.2 | 3.1–7     | 44.4     | 9       | 2         |
| Cow pea        | 23.5      | 1.3       | -        | -       | -         |
| Mung bean      | 22.9–23.6 | 1.2       | 45       | 7.0     | 1.1       |
| Pigeon pea     | 19.5–22.9 | 1.3–3.8   | 44.3     | 10      | 2.5       |
Table 2. Food products developed from pulses and legumes.

| Products                  | Description                                           | References |
|---------------------------|-------------------------------------------------------|------------|
| Soup                      | Vegetable soup supplemented with soy flour rich in protein and bioactive compounds | [3]        |
| Hummus                    | made from mashed chickpea and tahini                  | [37]       |
| Sweets                    | Sweet meats or baked product by using chickpea flour by using colors | [38]       |
| Snacks                    | By extruding a blends of chickpea and bovine lungs    | [39]       |
| Protein isolate           | By alkaline solubilization followed by isoelectric precipitation and freez drying from desi and kabuli chickpea | [40]       |
| Green gram Milk           | Rich in protein, vitamin C and dietary fiber          | [37]       |
| Noodle                    | Made from mungbean having good amount carbohydrates   | [13]       |
| Cowpea weaning food       | De-hulled, boiled cowpea supplemented to cereal-based infant foods | [37]       |
| Soy milk, Soy nuts, Soy yogurt, Tofu, Miso, Soy sauce, Natto, Tempeh, Soy protein isolate, Okara, Soybean oil. | All these products are made from soybean which contain high nutritional value and and some important isoflavones such as genistein, daidzein and glycitein, helps in preventing disease like breast cancer, cardiovascular disease, diabetes etc., | [42]       |

3.2. Mungbean

Mungbeans (Vigna radiata) is a vital pulse crop which has been grown worldwide mostly in Asia which accounts for 90% of production. India is the largest producer of mungbeans with 50% of world production followed by China and Myanmar [12].

3.2.1. Protein

Mungbean is a good source of protein and contains about 20–25% of total dry weight. In mungbean, large number proteins exist as storage proteins such as globulin (60%) and albumin (25%) [13]. It contains a high quantity of essential amino acids such as phenylalanine (1.443%), leucine (1.847%), isoleucine (1.008%), valine (1.237%), tryptophan (0.26%), arginine (1.672%), methionine (0.286%), lysine (1.664%), threonine (0.782%), and histidine (0.695%) [14,15]. Apart from these mungbeans also contains a significant amount of phenolic compound, Guo et al. [19], reported the total phenolic compound ranges from 2.04 - 2.24 mg GAE/g (GAE: Gallic acid equivalents) and flavonoids are in the range of 1.74–2.16 mg RUE/g (RUE: Rutin acid equivalents). Phytoic acid and polyphenols were found in the range of 734–806 mg/100g and 293–353 mg/100g as reported by Dahiya et al., [18] for different verities of mungbeans. The presence of phytic acid and polyphenol are found to be responsible for reducing the digestibility and bioavailability of nutrients present in mung bean [13].

3.2.5. Minerals

It contains 3.1–4% of ash [15,17,18] which shows that mungbean has appreciable amount of minerals. Minerals like iron, magnesium, sodium, potassium, calcium and zinc are found in the range of 3.4–4.4 mg/100g, 129–166 mg/100g, 8.7–13.2 mg/100g, 363–414 mg/100g, 81–114 mg/100g and 1.2–2.1 mg/100g respectively [18].

3.2.6. Bioactive compounds

It contains isoflavones, such as genistein (0.09mg/100g). Flavonols such as kaempferol (0.1mg), myricetin (0.1mg), and quer cetin (0.1mg) are present in it [15]. Apart from these mungbeans also contains a significant amount of phenolic compound, Guo et al. [19], reported the total phenolic compound ranges from 2.04 - 2.24 mg GAE/g (GAE: Gallic acid equivalents) and flavonoids are in the range of 1.74–2.16 mg RUE/g (RUE: Rutin acid equivalents). Phytic acid and polyphenols were found in the range of 734–806 mg/100g and 293–353 mg/100g as reported by Dahiya et al., [18] for different verities of mungbeans. The presence of phytic acid and polyphenol are found to be responsible for reducing the digestibility and bioavailability of nutrients present in mung bean [13].

3.3. Peas

Peas (Pisum sativum) are one of the most important crops which has been grown globally for the consumption of human and animal. Total global production of green peas and dry peas are 19 million tonnes and 14 million tonnes respectively. Canada is the largest producer of dry peas with 2.6 million tonnes followed by France (1.5 million tonnes), Russia (1.3 million tonnes), China (1.1 million tonnes) and India (0.7 million tonnes) [4].

3.3.1. Protein

Peas contain protein in the range of 21.2–32.9% and it largely varies due to environmental and genetic factor. Most of the protein present in it is storage proteins or globulins, and their nutritional values are determined by the amino acids profile. It contains a higher amount of arginine, valine, and methionine and lower amount of glutamic acid and cysteine as compare to soybeans and lupin. Presence of protease inhibitors in raw pea is responsible for low in vitro digestibility but still, its digestibility is higher than soybean and other pulses [20].
3.3.2. Carbohydrates
The amount of carbohydrate in whole peas ranges from 56.6 - 74.0 %, and in the kernel, it ranges from 62.8 - 78.6%. Starch is composed of two different types of glucose, i.e., amylose and amylopectin. Wrinkle peas contain lower level of starch (28-37%) than the smooth peas (44-46.3%) where as the amylose content is higher in the wrinkled peas (60-75%) than the smooth peas (20-38%). It has also been found that sucrose content in wrinkle peas is higher than the smooth peas [21]. The desirability of starch is directly influenced by the ratio of amylose and amylopectin and thus affecting the postprandial glucose response [20]. Peas also contain a good amount of insoluble carbohydrates, i.e., hemicelluloses (7.5%), lignin (1.4%), celluloses (68%) and crude fiber (58%) [21]. In a study, flour of whole yellow pea has been used to prepare the food of lower glycemic index and then compared with food prepared from flour of whole wheat. The result showed that postprandial glucose responses has reduced in individuals who consumed foods prepared by using flour of whole yellow pea and, thus, may prevent type 2 diabetes [51].

3.3.3. Lipids
Peas are low in lipids ranges from 0.8-6.1%. Total lipids present in the wrinkle seeds and round seeds are 4.5–5.2% and 2.8–3.1% respectively. Fatty acid composition of peas (g/100g total fatty acids) has been reported to be palmitic acid (8.6-19.5g); stearic acid (2.3–4.6g); oleic acid (14.2–33.3g); linoleic acid (38–60.9g); and linolenic acid (6.4-13.4g) [21].

3.3.4. Vitamins
The vitamins present in the raw peas per kg are ascorbic acid (22–295mg), thiamin (4.4–6.2mg), riboflavin (0.7–6.4mg), niacin (10.6mg), pantothenic acid (21.0mg), vitamin B-6 (1.0mg), pyridoxine (1.0mg), beta Carotene (7.0µg), biotin (8.2mg), folic acid (0.5mg), tocopherol (22–71mg), inositol (1.5mg) and vitamin K (1.5mg) [21].

3.3.5. Minerals
Peas are good source of many important minerals such as calcium (0.3–1.4 g/kg DM), potassium (7.2–12.5 g/kg DM), phosphorus (2.2–5.1 g/kg DM), Magnesium (0.9–2.6 g/kg DM), iron (22–490 mg/kg DM), zinc (20.4–63.5 mg/kg DM), sodium (29.5–1500 mg/kg DM), copper (4.9–10.1 mg/kg DM) and manganese (8.7–139 mg/kg DM). Most of these minerals are present between the testa and the kernel; the latter is richer in calcium and potassium [21]. Because of high phytate content in peas, the bioavailability of Zn, Fe, and Ca are very low [20].

3.3.6. Bioactive compounds
It contains a number of phytochemicals such as phenolic compounds, phytates, saponins, and oxalates. Saponins are strong surface active compound due to its amphiphilic nature. Till now, not much study have been conducted on hyperlipidemic or hypolipidemic action of saponins the results can be conflicting [58]. However, some studies on animals and in vitro models suggest that saponins posses anticiarcinogenic, antimutagenic, hypoglycemic, hypcholesterolemic, hepotoprotective, immunomodulatory, neuroprotective, anticoagulant, anti-inflammatory and antioxidant activities [59]. It has been also found that the excretion of bile acids has been improved by saponins, which helps in decreasing the level of cholesterol indirectly [58]. Therefore saponins have potential to play a key role as pharmaceutical and nutraceutical agents [59].

4. Health benefits of pulses and legumes

4.1. Soybean <!———->

Various studies on human population have shown decline in serum cholesterol, total cholesterol, LDL-cholesterol, and triglycerides due to intake of soy protein as compare with animal protein [22,23]. High isoflavones content in soybean helps in reducing the risk of several chronic diseases, increase bone density in women, inhibit bone resorption and inhibit growth of cancer cell [24,47-49], it also lowered the level of serum estrogen and prolonged menstrual cycle lengths by 20 min with the consumption of soy-based protein for two weeks [28]. It also reduces the risk related with cardiovascular diseases as well as decreases the incidence of cancer have also been found [24, 25]. Soy protein helps in lowering the Systolic and diastolic blood pressure [26,27]. Systolic and diastolic blood pressure in both hypertensive and normotensive women can be significantly reduced by consuming 25g soy protein per day [27,34]. There are various health benefits of dietary fiber such as reduction of toxic metabolites, prevention of pathogenic and autogenous diarrhea, constipation prevention, protection of liver function, lowering blood pressure, etc., [6,52, 53]. Soy consumption is also helpful in relief from menopausal symptoms [50]. Thus these attributes clearly show the relevance for food enrichment with soybean.

4.2. Mungbean

Phenolic acids play an important role in antioxidant properties, antimicrobial activity, and anti-inflammatory activities [29]. Quercetin, kaempferol, and myricetin are the flavonoids which possess antimutagenic, anticancer, and antihypertensive activities. There are several other health benefits of mungbeans like reducing risk of type 2 diabetes, obesity, heart diseases, high blood pressure, LDL cholesterol and many more that’s why consumption of mung beans has been seen snowballing along with cereals [29].
4.3. Peas

In a study, it was found that entire pea flour and fractioned pea flour reduces the fasting insulin levels by 13.5% and 9.8% respectively [54], food containing 4g of pea hull fiber significantly increase the bowel movement frequency by 7.5% [55]. Addition 5g of inulin along with 1.4–3.4g of pea hull fiber in snacks increase substantially the bowel movement frequency by 24% [56]. Consumption of bread with 17% pea hull fiber can reduce glycemic response. Pea fiber-enriched bread increased the duration of satiety [30]. Fiber-rich food is known for lowering blood pressure level, inflammation and improving serum lipid level [57] and cardiovascular health.

5. Biofortification in pulses and legumes

Biofortification is a method by which the nutritional value of crops can be enhanced with the help of breeding, transgenic techniques, or agronomic practices [60]. In recent decade not much work has been done on the biofortification of pulses and legumes as compare to rice, wheat and maize. However, biofortification is one of the feasible way to reduce malnutrition problem among undeserved and malnourished rural people in more cost efficient manner.

5.1. Bioavailability of protein

Legumes are rich in micronutrients, but they also contain many antinutrients which need to be minimized to improve the bioavailability of micronutrients. In a study, it was found that inter-specific breeding (crossing of two species from the same genus) of mungbean (methionine content 0.17 g/kg) with black gram (which has high methionine content 1.8–2.0 g/kg) significantly enhanced the quality of protein in mungbean. BC1F2 is the hybrid variety of mung bean and black gram that contain γ-glutamyl-S-methyl-cysteine and γ-glutamyl-methionine, a dipeptide found in mung bean and black gram respectively. Thus, the protein content of mung bean can be improved through inter-specific breeding. For increasing the methionine content in legumes, transgenic approaches for production of sulfur-rich crop of narbon bean (Vicia narbonensis), lupin (Lupinus angustifolius), and forage alfalfa (Medicago sativa) has already studied so far [16].

5.2. Bioavailability of iron (Fe) and zinc (Zn)

Bioavailability of iron and zinc ranges from 5-15% and 18–34% respectively of total intake and thus required a considerable amount of iron and zinc to compensate its low bioavailability, which presents a major challenge for the biofortification strategy [31]. Table 3 contains factors present in pulses which promote and inhibits micronutrients bioavailability. Presence of phytate in pulses and legumes are responsible for the low bioavailability of iron and zinc. Biehl et al. [33], reported that phytic acid form complex with Ca, Mg, Cu, Fe and reduces its solubility. Thavarajah et al., studied the effect of temperature on phytic acids in legume seeds and concluded that concentration of phytic acid and zinc is found more in areas having higher temperature regime (8.8 mg/g and 69 mg/kg, respectively) or for legumes with average temperature regime (6.7 mg/g and 61 mg/kg, respectively) and the same trend is found with Fe also (116 vs. 113 mg/kg). Thus phytic acid concentration decreases when seeds expose to low temperature. Above mentioned findings are important for developing new strategies of biofortification for reducing phytic acid in staple crops [61].

Zou, Tao, et al. [35], reported that biofortification of soybean sprout with a solution of ZnSO4 (10 or 20 μg Zn/ml) has significantly enhanced the quantity of zinc and also had good bioaccessibility. In another study, biofortification of soybean is done with different concentration of strontium ion (0.5mM–3.0mM). At concentrations up to 1.5mM, strontium stimulated plant growth by approximately 19.42% fresh weight (14.70% dry weight) and 22.62% fresh weight (22.66% dry weight) for the shoots and roots, respectively. Although concentrations above 2mM were showed toxic impacts. In vitro studies showed greater impact of strontium salts in treating osteoporosis related problem and absence of toxicity in animals and humans [36].

International Centre for Tropical Agriculture, Columbia (CIAT) has developed a biofortified bean (Phaseolus vulgaris L.) through breeding of crops containing iron up to 0.1 g/kg, a significant increase over traditional mung bean which contain 0.06g Fe/kg [31]. Ascorbic acid increases the absorption of iron (Fe) present in plant origin foods by forming Fe(III) complexes and reducing amount of Fe3+ to more soluble and bioavailable Fe2+. Therefore, ascorbate level in plant foods can be increased by using rDNA technology which would help to reduce the negative impact of phytate and polyphenols in staple foods on bioavailability of Fe and also make these foods as essential source of significant nutrient and vitamin C [64].

Similarly saponin, lathyrogens, protease inhibitor, and α-amylase inhibitors are also present in legumes which reduces the bioavailability of micronutrients. Saponin when consume in moderate quantity will provide various health benefits which has already discussed and consumption of saponin in large quantities can have various ill effect on health in the form diarrhea, vomiting and abdominal pain [63]. The traditional methods used for domestic food preparation and processing changes the saponin concentration originally present in pulses. These methods includes Soaking, sprouting, ordinary cooking [59].

6. Conclusion

Pulses have been consumed for thousands of years in various forms throughout the world. They contain a number of nutritional components such as protein, minerals, and vitamins which are very much essential for good human health. Apart from these it also contains certain phytotoxic chemicals such as polyphenol bioactive compounds (saponin, phytosterols etc.) and other metabolites which possess antioxidant activities and thus helps in decreasing the risk of various health diseases such as obesity, diabetes, hypertension, cancer, cardiovascular diseases. Since the population of world is increasing rapidly and micronutrient malnutrition (hidden hunger) has become a major challenge for the world especially for undeserved and malnourished rural people. Therefore, with the help of biofortification in pulses and legumes bioaccessibility of micronutrients can be enhanced.

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