FORMULATION OF PROTEIN AND ENERGY ENRICH NUTRITIOUS BAR FROM BIOFORTIFIED PROCESSED SOYBEAN FLOUR

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**Abstract**

*This study was conducted from October, 2022–March, 2023 at I.C. College of Community Science, CCS Haryana Agricultural University, Hisar, Haryana, India, to study the nutritional evaluation of soybean bar. In recent years, the demand for ready-to-eat snack products with improved nutritional properties and functionality has increased, due to convenience appeal and desirability, especially among children. In this study, three types of soybean bars were developed from soaked soybean flour incorporation with sorghum flour. The result of the proximate composition indicated that the type-III soybean bar developed with 100% soaked soybean flour had significantly higher moisture, crude protein, crude fibre, crude fat and ash content when compared with type-I and type-II soybean bars, developed with 20 and 10% sorghum flour, respectively. The total sugar content was significantly higher in type-II soybean bar compared to other types of soybean bars. A similar effect was observed for reducing and non-reducing sugar content. In vitro protein and starch digestibility were significantly higher in type-III soybean bar than of type-II and type-I soybean bar. The phytic acid content was significantly higher in the type-I soybean bar as compared to type-II and type-III soybean bars. The trypsin inhibitor content was not found in the Type-III soybean bar due to the NRC-127 soybean variety which is free from trypsin inhibitor content.*

**KEYWORDS:** *Soybean, sorghum, processing, nutritional evaluation, anti-nutrient factor, antioxidant*

**1. INTRODUCTION**

Soybean (*Glycine max* (L.) Merrill) is one of the most versatile legume crops globally. It is the source of protein (40–42%), edible oil (18–22%), carbohydrate, amino acids, fatty acids, vitamins (e.g. A, B, C and D) and antioxidants Anand et al. (2024). Soybean seed also contains 3% lecithin, which is required for brain development. The soybean seeds contain minerals such as: potassium, phosphorus, magnesium, calcium, and iron which are required for the vital functions of the body and play an important role in development of human health (Bagale, 2021). Soybean is one of the cheapest and nutritious products because of its unique chemical composition. It has the highest protein content among the cereal and pulses species (Garg and Brar, 2017). Soybean oil also contains omega-3 fats, which are beneficial for the heart, found in salmon and sardines, but the fat is less common in plant-based foods Deol et al. (2017). As a result, there is a growing demand for protein-rich foods in developing countries due to their affordability, availability, and accessibility to the population (Tao and Li, 2018).

The soybean seed comprises various essential isoflavones, namely, daidzein and genistein having medicinal properties. They have bioactive components having radical scavenging, anti-tumor, and anti-carcinogenic activity Chauhan et al. (2022). Due to the presence of bioactive components such as isoflavones, which help to lower blood serum cholesterol levels, cancer, osteoporosis, chronic renal disease, heart disease, oxidative stress and the risk of cardiovascular diseases (He and Chen, 2013). Antinutritional factors such as oxalates, phytates, tannin and saponin present in the soybean can interfere with the absorption of nutrients and reduce their bioavailability to human beings Joseph et al. (2024). Due to a decrease in nutrient bioavailability, anti-nutritional substances mix with nutrients to become the main cause of worry Samtiya et al. (2020). Soaking is a common domestic technique for hydrating the seeds and decreasing the anti-nutritional compounds in cereals and pulses by leaching in water Singh et al. (2017). According to (Gadzama, 2022) a long duration of soaking led to a greater reduction in anti-nutritional factors (ANFs). Soybean products are increasingly becoming popular especially amongst health-conscious people Sahana et al. (2020)**.** Snack bar is an alternative food made from cereals, pulses and other ingredients which is relatively practical, and easy to obtain, serve and consume Rahmi et al. (2021). Cereal bars have high nutritional value because they are prepared from cereals, dried fruits, raisins. They are good source of protein, energy, fiber, vitamins, antioxidants, etc. In addition, they are fortified by the use of a wide range of proteins such as soybeans, whey protein, grains, barley, vitamins and minerals Abdel-salam et al. (2022). Food bars are more resistant to pressure than biscuits/cookies because they are semi-wet food products Fatmah et al. (2021). Ready-to-eat products have garnered more attention among consumers since it is convenient and suits the palatability of the majority (Samuel and Peerkhan, 2020). Due to lack of time and fatigue drive people toward ready-made snacks, while higher energy and protein needs increase food intake. For athletes, small easily digestible meals make supplements and functional foods the best option Malecki et al. (2022).The lifestyle changes increased the consumption of snacks and fast food, leading to demand for its production has increased. Snacks enriched with fibers and antioxidants from fruits are seen as healthy and convenient Abbas et al. (2025). Therefore, keeping in view the above facts, the present study has been carried out with the following objectives: 1) develop the soybean bars using processed soybean flour; 2) assess the nutritional composition of soybean bar made from processed soybean flour.

**2. Material and Methods**

***2.1.Study area***

The study was conducted from October, 2022–­­­­March, 2023 at I.C College of Community Science, CCS Haryana Agricultural University, Hisar, Haryana, India.

***2.2.Material***

In the present study develop soybean bar using biofortified variety NRC-127 procured from ICAR-Indian Institute of Soybean Research, Indore. The locally available variety of sorghum i.e., HC 308 was procured from Forage section, Department of Genetic and Plant Breeding, CCSHAU, Hisar. Other ingredients like peanut butter, chocolate, honey, almond, cashew nut, pistachios, flax seed, pumpkin seed, sesame seed, cranberry and blue berry used in the preparation of soybean bars were procured from local Hissar market in a single lot.

***2.3.Processing of soybean***

Soybean seeds were cleaned and sorted to removing stones, foreign particle and other physical contamination. The soybeans were soaked in water for 12 hours at 370C. After draining they were dried in a hot air oven and then ground with a mixer. The flour obtained was sieved and packed in low density polythene bags and stored for further analysis Ouedraogo et al. (2022).

***2.4.Preparation of soybean bar***

Soybean bars were developed in the laboratory of Foods and Nutrition, I.C College of Community Science, CCS HAU, Hisar. Bars were developed using main ingredients as processed soybean flour with sorghum flour. Three different types of bars (I, II, III) were prepared with proportion of 80:20 (soybean+sorghum) 90:10 (soybean+sorghum) and 100% processed soybean flour. (Table 1). Processed soybeans were ground with a mixer grinder and obtain fine powder. Almond, pistachio and cashew were roasted and powdered coarsely. Sesame seed, flax seed and pumpkin seed were also roasted and make coarse powder. Peanut butter and chocolate were put on a flame and they were melted. To it soybean flour, Almond, pistachio, cashew powder and powdered of sesame seed, flax seed and pumpkin seed were added and mixed well. The mixture was cooled and rolled to desirable thickness. The mixture was cut into desirable shape of bars and was wrapped in butter paper. The prepared bars were subjected for nutritional evaluation (Figure 1) show the different type of soybean bar made from processed soybean flour.

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| Table: 1 Ingredients used for making three type of soybean bar | | | |
| **Ingredients** | **Soybean bar (Amount)** | | |
| Type-I | Type-II | Type-III |
| Processed soybean flour (g) | 80 | 90 | 100 |
| Sorghum flour (g) | 20 | 10 | - |
| Peanut butter (g) | 30 | 30 | 30 |
| Chocolate (g) | 40 | 40 | 40 |
| Honey (g) | 5 | 5 | 5 |
| Cashew (g) | 5 | 5 | 5 |
| Pistachio (g) | 5 | 5 | 5 |
| Almond (g) | 5 | 5 | 5 |
| Sesame seed (g) | 2 | 2 | 2 |
| Pumpkin seed (g) | 2 | 2 | 2 |
| Flax seed (g) | 2 | 2 | 2 |
| Cran berry (g) | 2 | 2 | 2 |
| Blue berry (g) | 2 | 2 | 2 |
| Vanilla essence | 2 drops | 2 drops | 1. drops |



**Figure 1:** Different type of soybean bar made from processed soybean flour

***2.5.Chemical analysis***

***2.5.1.Proximate analysis***

Moisture, crude fat, crude protein, crude fiber and ash contents of processed soybean were determined according to the methods of the (AOAC, 2000). Carbohydrates were calculated by the difference method. Energy of processed soybean bars were calculated by factorial method by multiplying the crude protein, carbohydrate and crude fat in the sample by 4, 4 and 9 kcal/g, respectively using the formula

Energy (Kcal 100/gm)=4.0×protein (%)+4.0×carbohydrate (%)+9.0×fat (%)

***2.5.2.Determination of available carbohydrate***

Total sugar and reducing sugars were determined using Yemm and Wills (1954) and Somogyi (1945) respectively. Non reducing sugars were calculated by difference between total and reducing sugars. Starch was determined using the method of Clegg (1956).

***2.5.3.In vitro protein and starch digestibility***

*In vitro* protein digestibility was carried out by the modified method of (Mertz et al., 1983). *In vitro* starch digestibility was assessed by (Singh et al., 1982).

***2.5.4.Evaluation of anti-nutritional factor of bar***

Phytic acid was determined by the method of Haug and Lantzsch (1983). Trypsin inhibitor activity was determined by the modified method of Roy and Rao (1971).

***2.5.5.Analysis of bioactive component***

The concentration of total phenolics of the methanolic extracts was determined by the Folin-Ciocalteau colorimetric method (Singleton and Rossi, 1965). The DPPH free radical scavenging activity of sample extracts was evaluated by the DPPH method of (Hatano et al., 1988) and total antioxidant activity was evaluated by (Prieto et al., 1999).

***2.5.6.Statistical analysis***

The data obtained in the present investigation were subjected to analysis of variance (ANOVA) techniques and analysed according to three three-factorial completely randomized design (C.R.D). The critical difference value (*p*<0.05) level was used for making comparison between different types of developed products.

**3. Results and Discussion**

***3.1. Proximate composition of soybean bars***

The result of the proximate composition of soybean bar made from soaked soybean flour and sorghum has been presented in (Table 2) indicated that the moisture content of type-III soybean bar was 4.20%, which was found higher significantly (*p*≤0.05) than that of type-I soybean bar 3.56% and type-II soybean bar 2.59%. The result of present finding is supported by earlier worker Joshi and Verma (2016) reported moisture content of the flour samples (4.1–10.5 g 100/g) are within the values (10–13 g 100/g) considered low enough to ensure the safety of the flours during storage. Our similar findings have been reported by Rahmi et al. (2021) that the fat content of commercial bar and composite of sweet potato and soybean flour bar was 24.73 and 18.29% respectively. Carbohydrate content of type-II soybean bar and type-III soybean bar were found to be 35.71 and 34.62% which did not differ significantly but both had significantly lower carbohydrate content than that of type-I soybean bar 43.55%. Energy (kcal) content of type-II soybean bars (537.33%) had significantly higher energy (kcal) content than that of type-I soybean bar 500.40% and type-III soybean bar 519.62%. Crude protein content of type-I soybean bar, type-II soybean bar and type-III soybean bar were found to be 17.73, 17.38 and 18.90%, respectively, which were almost similar. Crude fat content of type-II soybean bar (36.10%) and type-III soybean bar (34.26%) which did not differ significantly but both had significantly (p≤0.05) higher crude fat content than that of type-I soybean bar (28.36%). Balance energy-protein in pregnancy as much as 12.3% energy from protein can increase fetal growth. The results of the present study are supported by the findings of earlier worker, Padmashree et al. (2012) reported the protein contents of the bars between 7.41 and 18.8%. Crude fibre content of type-I, type-II bar and type-III soybean bar were found to be 4.10, 5.13 and 4.82%, respectively, which did not differ significantly.

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| Table 2: Proximate Composition of soybean bars (%, on dry matter basis) | | | | |
| Soybean bar  Parameter | Type-I | Type-II | Type-III | CD (p≤0.05) |
| Moisture\* | 3.56±0.12b | 2.59±0.26c | 4.20±0.11a | 0.61 |
| Crude protein | 17.73±1.11a | 17.38±0.11a | 18.90±0.72a | 2.66 |
| Crude fat | 28.36±0.78b | 36.10±0.79 a | 34.26±0.93 a | 2.91 |
| Crude fibre | 4.10±0.41a | 5.13±0.45a | 4.82±0.20a | 1.29 |
| Ash | 3.10±0.02 a | 3.07±0.04 a | 3.253±0.10 a | 0.23 |
| Carbohydrate | 43.55±0.89a | 35.71±0.72b | 34.62±1.70b | 4.11 |
| Energy (kcal) | 500.40±6.30b | 537.33±5.74a | 519.62±6.88ab | 21.89 |

Values are mean±SE of three independent determination

Similar superscripts in the column indicate that they do not differ significantly (*p*≤0.05)

\* On fresh basis

NS- Non significant

***3.2. Available carbohydrate of soybean bar***

The results given in (Table 3) show that total sugar content of type-I bar and type-III soybean bar were found to be 13.92 and 13.80 g 100/g respectively, which did not differ significantly but type-II soybean bar had higher total sugar content (15.32 g 100/g). The type-III soybean bar had higher reduced sugar content than type- II and type-I soybean bar. A significant reduction of non-reducing content was observed in type-I soybean bar. In comparison to the type-I bar (50.57g 100/g), type- II and type-III soybean bar had significantly (*p*≤0.05) lower starch content 48.56 and 46.87 g 100/g respectively.

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| Table 3: Carbohydrate content of soybean bar (g 100/g, on dry matter basis) | | | | |
| Soybean bar  Parameter | Type-I | Type-II | Type-III | CD (p≤0.05) |
| Total sugar | 13.92±0.20b | 15.32±0.21 a | 13.80±0.12b | 0.64 |
| Reducing sugar | 8.42±0.12b | 8.82±0.07a | 7.30±0.13c | 0.39 |
| Non- reducing sugar | 5.50±0.19b | 6.49±0.22 a | 6.50±0.15 a | 0.67 |
| Starch | 50.57±0.12 a | 48.56±0.16b | 46.87±0.10c | 0.45 |

Values are mean±SE of three independent determination

Similar superscripts in the column indicate that they do not differ significantly (*p*≤0.05)

NS-Non-significant

***3.3. In vitro protein and starch digestibility of soybean bar***

The data related to *In vitro* protein and starch digestibility indicated in (Table 4). *In vitro* protein digestibility of type-III soybean bar was significantly higher as compared to type-II soybean bar, although there was no significant difference between type-I and type-III soybean bar. The research finding of Khetarpaul and Chauhan (1990) reported that the *In vitro* starch digestibility increased by more than three-fold when pearl millet grains were germinated for 24 h. *In vitro* protein digestibility of pearl millet grains was 77.2%. *In vitro* starch digestibility content of type-II soybean bar (44.96 mg maltose released/g) which did not differ significantly to type-I soybean bar (44.73 mg maltose released/g) and type-III soybean bar (45.96 mg maltose released/g).

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| Table 4: *In vitro* protein (%) and starch (mg maltose released/gmeal) digestibility of soybean bars (on dry matter basis) | | | | |
| Soybean bar  Parameter | Type-I | Type-II | Type-III | CD (p≤0.05) |
| Protein | 70.04±0.09 a | 68.89±0.25b | 70.60±0.28 a | 0.79 |
| Starch | 44.73±0.21b | 44.96±0.03ab | 45.96±0.49a | 1.07 |

Values are mean±SE of three independent determination

Similar superscripts in the column indicate that they do not differ significantly (*p*≤0.05)

NS-Non-significant

***3.4. Anti-nutritional factor of soybean bar***

The data presented in (Table 5) indicated the anti-nutritional factor of soybean bars made from processed soybean flour i.e. Type-I, Type-II and Type-III. Phytic acid content of type-II soybean bar 2.33 mg/gand type-III soybean bar (2.28 mg/g) varied non-significantly from each other. However, type-I soybean bar had significantly higher i.e. 2.83 mg/g of phytic acid in comparison to type-II and type-III soybean bars. In soaking resulted in loss of phytic acid significantly. Trypsin inhibitor content of type-I soybean bar (16.42 mg/g) had significantly (*p*≤0.05) higher trypsin inhibitor content than that of type-II soybean bar (12.17 mg/g) but type-III soybean bar had no trypsin inhibitor contents. A study done by (Gilani et al., 2005; Coulibaly et al., 2011) reported that various processing techniques including dehusking, sprouting, blanching, milling, soaking, fermentation, cooking and baking are reported to promote reduction of anti-nutritional factors in food materials. Germination was also resulted in decreased the phytic acid and a similar trend was observed by (Afify et al., 2011).

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| Table 5: Phytic acid (mg/g) and trypsin inhibitor content of soybean bar (on dry matter basis) | | | | |
| Soybean bar  Parameter | Type-I | Type-II | Type-III | CD (p≤0.05) |
| Phytic acid | 2.83±0.06 a | 2.33±0.06b | 2.28±0.02b | 0.19 |
| Trypsin inhibitor | 16.42±0.09 a | 12.17±0.09b | ND | 0.38 |

Values are mean±SE of three independent determination

Similar superscripts in the column indicate that they do not differ significantly (*p*≤0.05)

NS-Non-significant

***3.5. Bioactive component of soybean bar***

Table 6. show that DPPH free radical scavenging activity, total antioxidant activity, total phenolic content total flavonoid content of type-III soybean bar was found to be significantly higher than type-I and type-II soybean bars. DPPH free radical scavenging activity content of soaked type-I, soaked type-II and soaked type-III were found to be 134.54, 135.12 and 139.64%, respectively which did differ significantly from each other. The use of processed soybean flour resulted in increased of antioxidant activity of soybean bar. The total phenolic content of type-I, type-II and type-III soybean bars were found to be 3.56, 3.68 and 3,83 mg/g, respectively which did differ significantly each other. The total Phenolic content of raw chickpea and lentil seeds, ranging from 0.98 to 47.6 mg GAE/g (Han and Baik, 2008; Xu et al., 2007; Zhao et al., 2014; Wang et al.,2016; Xu and Chang, 2012) reported that the antioxidant activity of common bean-based snacks measured by DPPH radical inhibition ranged from 1.6 to 6.17 μmol TE/g. Those values are within and below the range of the antioxidant activity found in raw common beans (5.26–19.4 μmol TE/g).

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| Table 6: DPPH free radical scavenging activity (%), total antioxidant activity (mg/g), total phenolic content (mg/g) and total flavonoid content (mg/g) of soybean bar (on dry matter basis) | | | | |
| Soybean bar  Parameter | Type-I | Type-II | Type-III | CD (p≤0.05) |
| DPPH | 134.54±0.06c | 135.12±0.05b | 139.64±0.11a | 0.28 |
| TAA | 11.66±0.06b | 12.96±0.03 a | 13.08±0.07 a | 0.20 |
| TPC | 3.56±0.01c | 3.68±0.00b | 3.83±0.02a | 0.06 |
| TFC | 0.55±0.01b | 0.77±0.00 a | 0.81±0.00 a | 0.03 |

Values are mean±SE of three independent determination

Similar superscripts in the column indicate that they do not differ significantly (*p*≤0.05)

NS-Non-significant

**4. Conclusion**

This study concluded that the soybean bars were found to be high in energy with good amounts of crude fibre and protein. Among all types of bars, the type-III soybean bar was found to be high in crude protein, fat, fibre and low in phytic acid. The soybean contained no trypsin inhibitor content. Hence, the developed bar was recommended to improve the nutritional status of school-going, homemakers and sports persons.

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