ABSTRACT

Black soybean (*Glycine max (L.) Merrill*) locally known as *bhat, kalabhat or bhatmaas* is an underutilized leguminous crop confined to the Indo-Himalayan regions. Black soybean has unique properties owing to its black hull, making it different from yellow and green soybean. There is wide variability in the *black soybean* in terms of seed weight, length and width and other quality parameters. There is a need to explore these legumes to assess their genotype diversity for further utilization in food and seed improvement programs. With this background, the present research was designed to validate the nutritional claim in the Indo-Himalayan *black soybean* population, its lipid quality assessment, effect of cooking methods on antinutritional factors, and development of value-added products including its nutritional quality and sensory evaluation.

Black soybean specimen samples from five geographical locations of Indo-Himalayan regions (BSALS & BSALB, BSNDD, BSRMN and BSGRP - samples collected from Almora, Dehradun, Ramnagar and Nainital regions, respectively) were collected. The samples were authenticated and analyzed in terms of seed dimensions and proximate, mineral and amino acid compositions to evaluate the seed variability. Polyphenolic compounds extracted using a central composite design by response surface methodology (RSM) using ultra-sound assisted (UAE) extraction techniques with three process variables (solid to solvent ratio, ultrasound amplitude and time) and three responses i.e. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, total phenolic content (TPC) and total flavonoid content (TFC). The UAE optimized samples were analyzed to target three β-glucoside, three aglycon isoflavones, one anthocyanin cyanidin-3-glucoside and three polyphenols (gallic, caffeic and quercetin) using HPLC technique. The lipid quality was assessed among the samples for oil yield, β-carotene, tocopherols, and unsaponified matter. Fatty acid and flavor estimation was performed using GC and GC-MS-HS. Supercritical fluid and Soxhlet extractions were compared for different oil quality characteristics of *black soybean*. Effects of microwave cooking (400, 800 and 1200 W for 10 min), pressure vessel (48.26 kPa, 68.95 kPa, and 82.73 kPa, respectively), and conventional clay pot cooking at 70°C, 90°C and 110°C on raffinose and phytic acid - two antinutrients in *black soybean* were analyzed. Further, black soybean flour was utilized for the preparation of cookies.

There was a significant variation ($p<0.05$) in the seed weight (0.084 to 0.158 g) among the seed samples. The BSALS seeds had the lowest width and weight comparatively. The protein content in all samples were measured to be in the range 31.1 to 39.7 g/100g with the maximum in small oval shape BSALS seeds, a nonsignificant difference ($p>0.05$) among the black soybean samples revealed the variance in the proximate compositions irrespective of the seed dimensions. Sucrose was observed in the range of 3.84 to 5.02 g/100g with a minimum concentration in BSALS and maximum in BSGRP seeds. FTIR characterization reported three major peaks at 2835, 1640, and 1015 cm^{-1} wavenumber and reported the presence of aldehyde and ketone compounds. Protein quality assessment for individual amino acids (AAs) reported sixteen AAs, of which seven were classified as essential and nine as non-essential AAs. On differentiating the AA values with the physical characteristics of *black soybean* varieties, it was found that BSGRP samples possessed the minimum AA and the maximum was noted in BSALS samples. Among essential AAs, lysine was comparatively higher in BSALS seed samples (2.32 g/100g). Sulphur containing amino acid methionine and

cysteine had low levels in all samples. Co and Cr were not detected in any of the samples among eleven mineral elements. Micronutrients were comparatively higher in BSALS seed samples.

The UAE extraction conditions (ultrasound power, time and solid-to-solvent ratio) for polyphenols (DPPH assay, TPC and TFC) were optimized using a central composite design by response surface methodology. A quadratic model with F-values of 29.38 and 8.77 was suggested for DPPH assay and TPC, respectively. The extract optimized using UAE process were analyzed using HPLC-UV for estimation of isoflavones and anthocyanin content. Total isoflavones significantly ($p<0.05$) varied from 85.41 to 199.44 mg/100g with the highest concentration in BSALS samples. β-glucoside isoflavones were reported 85.73 to 93.18% of total isoflavones. Cyanidin-3-glucoside (C3G) varied from 29.23 to 71.85 mg/100 g with the maximum in BSGRP seed samples and lowest in BSALS samples. The BSALS samples had a lower oil content (about 20.53%), and maximum β-carotene content (1305 µg/kg), while the oil yield was about 25.64% in BSALB seed samples. γ-tocopherol was highest, followed by δ- tocopherol and α-tocopherol among all seed samples. The oil was consisted of five major fatty acids: methyl palmitate (9.79 to 10.87%), methyl stearate (3.12 to 4.26%), cis 9-oleic acid methyl ester (19.46 to 25.4%), methyl linoleate (54.63 to 57.44%) and, methyl linolenate (5.81 to 9.75%) with the non-significance distribution. Lipid fractions reported 46 volatile flavor compounds (VFCs) as identified in GC-MS/HS analysis. There was a wide difference in the composition of these compounds among individual lipid fractions. Supercritical fluid extraction (SFE) reported oil yield of 19.1%, while the Soxhlet produced 23.67% oil yield. Unsaponified matter and carotenoid content in SFE technique were slightly higher (2.73% and 1.259 mg/kg) than the Soxhlet extraction (2.57% and 1.139 mg/kg), indicating that SFE oil exhibited comparatively higher antioxidant activities. Flavor 1,2-Propanediol, 3-benzyloxy-1,2-diacetyl- was observed higher in SFE.

The conventional clay pot and pressure vessel cooking methods reported to reduce about 32.50% and 75.0% of phytic and raffinose, respectively, while cyanidin-3-glucoside was significantly degraded in all the treatments. *Black soybean* flour-based gluten-free cookies were prepared to realize the consumers perspective for the black soybean derived food product. The average sensory score for flavor and color were 7.8 and 8.2. The texture, taste, and mouthfeel scores were 8.1, 8.0 and 8.1, respectively on a 9-point hedonic scale and showed good consumer acceptability.

In conclusion, the present study reports the scientific validation of local *black soybean* for phytochemical and lipid composition. This profiling would help developing various value added and protein rich nutritious food products exploring utilization of underutilized *black soybean* in local and global markets.

Keywords: Antinutrients; *Black soybean*; Lipid characteristics; Antinutritional factors; Sensory qualities; Ultrasound assisted extraction