Color based genotypic differences effect the techno-functional and bioactive properties of common bean (*Phaseolus vulgaris* L.) protein concentrates

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Research Article

Keywords: Common beans, genotype, protein extraction, salt, bioactivity,

Posted Date: May 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-478103/v1>

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Abstract

In this study, technofunctional and bioactive properties of protein concentrates from different common bean genotypes were investigated. Protein extraction conditions depending on pH and salt level were optimized by response surface methodology. The highest protein content for the concentrates (77.7%) was determined for pH 11 and 0.4% of salt. The protein content of the common bean flour and their protein concentrates were in the range of 22-26.96% and 72.97–77.99%, respectively. For bioactive properties, total phenolic content ranged between 578.9-1355.9 and 313.6-1219.1 mg GAE/kg, for flours and concentrates respectively. Two genotypes (G7 and G8) were the samples showing the superior biofunctional properties. Principal component analysis showed that G2 had different characteristics in terms of technofunctional parameters while G7 and G8 were differed from the other genotypes in terms of bioactivity. The results revealed that the proteins of common beans could be evaluated as good source for the enrichment of food formulations.

1. Introduction

Dietary protein intake is one of the most important issue in daily diet because proteins play critical roles in human health with the functions of enzyme, antibody, hormone, and structural component. Recommended dietary allowance (RDA) for protein for adults is currently $\sim 0.8 \text{ g kg}^{-1} \text{ d}^{-1}$ [1]. Protein intakes in developed countries are mainly supplied from animal sources [2] however, plant protein ingredient market is expected to grow faster to meet the demands of consumers with different ethnic, religious, dietary, and moral preferences [3]. Lonnie et al. [4] stated that plant-based nutrition has received much attention in the past decade with the effect of certain health disorders such as excess body weight, diabetes, heart attack, cancer, as a result of consumption of animal proteins.

Common beans (*Phaseolus vulgaris* L.) are economically, and nutritionally important edible seeds cultivated and consumed around the world [5]. Beans contain important compounds that may exhibit biological activities such as phenolic compounds, tocopherols, peptides, amino acids, dietary fiber, resistant starch, unsaturated fatty acids, and minerals [6]. It was reported that regular intake of dried beans has reduced glycemic index and low-density lipoprotein (LDL) while increased high density lipoprotein [7]. Besides several studies investigated the relation of between common bean consumption and cancer prevention [8, 9].

In addition to health effects, bean proteins can be used in food formulations because of their solubility, emulsifying, foaming, and water/fat-binding properties [10]. The determination of the functional properties of bean hydrolysates is very important and also growing location and genotype, and genotype $\times$ environment interaction, may influence the phytochemical composition of common beans [11]. In the present study, it was aimed to select the bean genotypes owing to different morphological characteristics in terms of color, shape and size and to investigate the technological and bioactive properties of their concentrates. To achieve that aim, response surface methodology was used to determine the optimum conditions for protein isolation process. The current study is the first in terms of using such a different genotype variation to our knowledge.

2. Material And Methods

2.1 Materials

Different common bean genotypes were provided from Agricultural Faculty of Kahramanmaraş Sütcü İmam University, Kahramanmaraş (Turkey) and the images of the bean samples were illustrated in Fig. 1. All methods were carried out in accordance with the relevant institutional, national, and international guidelines and legislation.

2.2. Protein extraction and optimization of processing variables

Before starting to the protein extraction from common been genotypes, two important processing variable levels, pH and salt concentration, were optimized. For this purpose, central composite rotatable design was used. The salt level was in the range of 0-0.4% while the pH ranged between 9–11. To determine the effect of salt and pH, different levels of these parameters were used for the protein extraction (Table 1).

Table 1
Central composite rotatable design to determine the optimum level for the processing variables

Runs	Salt (%) (X_1)	pH (X_2)	Protein Content (%)
1	0.2	9	69.52
2	0.4	10	72.34
3	0.2	10	70.94
4	0	9	73.70
5	0	11	62.00
6	0	10	67.94
7	0.4	9	68.58
8	0.2	10	69.98
9	0.2	11	69.87
10	0.4	11	79.03
11	0.2	10	70.80

For the extraction of proteins, 10 g of powdered common bean was added to 200 ml of distilled water containing a certain amount of salt (0-0.4%) and the suspension was mixed for 5 min. Then the pH of the sample was increased and adjusted the corresponding level in the experimental design (Table 1) by using NaOH (1 M) and, at the constant pH level, the samples were mixed for 1 hour at 1000 rpm using magnetic stirrer. After that, the slurry was exposed to centrifugation to separate the aqueous part from the residual part at 7500 g and 4°C for 3 min. The supernatants were collected in a beaker. This liquid part, in which the protein is in solution, was subjected to isoelectric precipitation by adding 0.1 M HCl until the pH value reaches 4.5. As soon as the pH value was exactly 4.5, the addition of HCl was discontinued and the sample was mixed in the magnetic stirrer for a further period. Then, the suspension was centrifuged at 8500 g and 4 °C for 3 minutes to collect the precipitated proteins and the precipitate was separated and finally washed for times by distilled water and neutralized. At the end of this extraction process, the samples were lyophilized and ground using a grinder for further analysis.

The protein content of the extracted protein concentrates was analyzed, and the levels were used as response to the relevant trial pattern and the optimum pH value and salt amount were determined using optimization procedure based on desirability function. After the determination of the optimum pH level and salt concentration, all common bean genotypes were exposed to protein extraction by following the process mentioned above. The extracted proteins were used to further technological and bioactivity analyses.

2.3. Analysis of some physicochemical and functional properties

2.3.1. Determination of protein content

Protein content of the common bean genotypes was determined by Dumas method using an automatic nitrogen analyzer (NDA 701 Dumas Nitrogen Analyzer, Velp, Italy) and the analyzed N content was multiplied by 6.25 for the calculation of total protein level.

2.3.2. Determination of color properties

Color properties of the samples were analyzed by using a Minolta automatic color analyzer the color characteristics were given as L (brightness), a (redness) and b (yellowness).

2.3.3. Solubility of the protein concentrates

For solubility analysis, 50 mg protein concentrate was weighed, and 10 mL distilled water was added. Samples were left in automatic vortex mixing for 1 hour at room temperature and then centrifuged at 8000 g for 15 minutes at room temperature. Then, 5 mL of solution from the upper phase in the tube was weighed and the amount of dry matter was determined by drying in the oven and the solubility values of the samples were determined from the residue weight on after drying [12].

2.3.4. Water and oil holding capacities (WHC and OHC)

WHC and OHC of the protein samples were determined according to Sciarini et al. [12]. Firstly, the empty eppendorf tube was tared and then 50 mg of protein concentrate was weighed into this eppendorf tube. Then, 1.5 mL of pure water was added, and the samples were vortexed for 20 seconds and kept at room temperature for 30 minutes, and the supernatant was discarded by centrifuging at 15,000 × g, 20 minutes, 4°C. The tube was then weighed again, and the calculation was performed using the formula below;

$$\text{WHC (g/g)} = (\text{W}_{\text{tube+residue}} - \text{Weight}_{\text{tube}}) / \text{Weight}_{\text{Sample}}$$

For OHC, 50 mg of protein concentrate was weighed into this eppendorf tube. Then, 1.5 mL of sunflower oil was added and the samples vortexed for 20 seconds were kept at room temperature for 30 minutes, after the centrifugation at 15,000 × g, 20 minutes, 4°C, the upper phase was discarded and then the

tube was weighed again and the calculation was made with the following equation;

$$\text{OHC (g/g)} = (\text{Weight}_{\text{tube+residue}} - \text{Weight}_{\text{tube}}) / \text{Weight}_{\text{Sample}}$$

2.3.5. Emulsion capacity and emulsion stability (EC and ES)

To determine the emulsion capacity values of protein concentrates, 0.25 g of protein powder was mixed with 5 mL of distilled water and the pH of this mixture was adjusted to 7 with 1 N NaOH. Then 5 mL of sunflower oil was added and homogenized for 1 minute at 10000 rpm. After the tubes were centrifuged at 3000 g for 15 minutes, height of emulsion layer (mL) and total tube volume (mL) were recorded. Emulsion capacity (EC) was calculated using the equation below. The tubes are incubated for 30 min at 80 ° C. After standing in a water bath, rapid cooling was done under running water and again centrifuged at 3000 g for 15 minutes. After centrifugation, emulsion stability (ES) was calculated using the second equation from the remaining emulsion layer (mL) and total volume (mL);

$$\text{EC (\%)} = (V_1/V_3) \times 100$$

$$\text{ES (\%)} = (V_2/V_3) \times 100$$

V_1 : Emulsion layer (mL)

V_2 : Emulsion layer after incubation (mL)

V_3 : Total tube volume (mL)

2.4. Analysis of bioactive properties of common bean flour and protein concentrates

2.4.1. Extraction of the samples

Bioactivity analysis was conducted for both common bean flour and protein concentrates. For all samples, extraction was performed with 80% methanol added with 0.1% HCl. For this purpose, 1 g sample of flour was treated with 20 mL of solvent and after 1 min vortexing, it was left for extraction in a shaking water bath for 1 h. At the end of the period, the samples were centrifuged at 9000 g and 4 °C for 3 min and the upper phase was used for analysis by passing the 0.42 µm syringe filter. In protein concentrates, 0.25 g of sample was treated with 1.75 mL of solvent, followed by extraction in a shaking water bath for 1 h after vortexing for 1 min. At the end of the period, the samples were centrifuged at 9000 g and 4 °C for 3 minutes and the upper phase was passed through a 0.42 µm syringe filter and used for analysis.

2.4.2. Determination of total phenolic content (TPC)

The total phenolic content of the samples was determined according to the methods of Singleton and Rossi [13]. For this purpose, 200 µL of extract was mixed with 1800 µL of distilled water and then 1 mL of Folin Ciocalteu (diluted 1/10 with water) reagent was added into the samples. Then, 2 mL of sodium carbonate (2% w / v) was incorporated into the samples and after the tubes were tightly closed, they were vortexed and incubated for 2 h at room temperature and in the dark conditions. At the end of the duration, the absorbance values of the samples were measured with a UV-vis spectrophotometer (UV-vis 1800, Shimadzu, Japan) at 765 nm. Total phenolic content was calculated as mg GAE/kg using the calibration curve prepared with gallic acid standard.

2.4.3. Determination of total flavonoid content (TFC)

The total flavonoid content of the samples was determined by the suggested method of Zhishen et al. [14]. For this purpose, 500 µL of extract was mixed with 2 mL of water and then 150 µL of NaNO₂ (5% w/v) was added to the samples. After 5 min, 150 µL of AlCl₃ (10% w/v) was placed into the tubes and after incubation for 6 minutes, 1 mL of NaOH was added and the sample volume was completed to 5 mL with the addition of 1.2 mL of water finally. After mixing the samples with vortex, they were incubated for 10 min at room temperature and in the dark conditions, and the absorbance values of the samples were measured with a UV-vis spectrophotometer (Shimadzu, Japan) at 510 nm. Total flavonoid content was calculated as mg CE/kg by using calibration curve prepared with catechin standard.

2.4.4. Determination of DPPH radical scavenging activity

DPPH radical scavenging activity of the samples was performed according to the methodology suggested by Brand-Williams et al. [15]. For this purpose, 100 µL of extract was mixed with 3900 µL DPPH solution (0.1 mM in methanol) and after vortexing, it was incubated for 30 min at room temperature and in the dark conditions. At the end of the period, the absorbance values of the samples were recorded with a spectrophotometer (UV-vis 1800 Shimadzu, Japan) at 517 nm and calculated as % inhibition using the following equation;

$$\% \text{ Inhibition} = ((A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}) \times 100$$

where A_{Control} is the absorbance value of the sample and A_{Sample} represents the sample absorbance value.

2.4.5. Determination of ABTS.⁺ radical scavenging activity

ABTS.⁺ radical scavenging activity of the samples was performed according to the methodology suggested by Gong et al. [16]. For this purpose, a stock solution of ABTS.⁺ was prepared by dissolving a certain amount of ABTS.⁺ radicals (Sigma, St. Louis, MO, USA) in 2.45 mmol/L potassium persulfate and stored for 16 h in the dark conditions at room temperature. At the end of the process, the stock solution was diluted with a certain level of phosphate buffer and its absorbance was adjusted to be 0.7 ± 0.05 at 734 nm. It was then mixed with 30 µL of extract with 2 mL of ABTS.⁺ solution and incubated for 6 min.

Finally, the absorbance value was measured with a spectrophotometer at 734 nm (UV-vis 1800 Shimadzu, Japan) and calculated as % inhibition using the following equation;

$$\% \text{ Inhibition} = ((A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}) \times 100$$

The % inhibition values were then expressed as a sample of μg Trolox (TE)/g with the aid of the Trolox calibration curve.

2.4.6. Determination of ferric reducing antioxidant activity (FRAA)

Ferric reducing antioxidant activity of the samples was determined according to the methodology suggested by Oyaizu et al. [17]. One mL of sample concentrates and standard (ascorbic acid) substance of various concentrations was mixed with 2.5 ml of 0.2 M phosphate buffer solution (pH = 6.6) and then 2.5 ml of 1% w/v potassium ferricyanide $[\text{K}_3\text{Fe}(\text{CN})_6]$. It was left to incubate for 20 minutes at 50 °C. After this step, 2.5 ml of trichloroacetic acid (10% w/v) was added to the reaction mixture and it was centrifuged for 10 min at 1000 g and 2.5 ml was taken from the top of the solution and 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl_3 were added, and the final mixture were vortexed and absorbance was recorded at 700 nm with a UV-vis spectrophotometer (Shimadzu, Japan). Results were expressed as mg ascorbic acid equivalent (mg AAE/kg).

2.4.7. Determination of antioxidant activity (AA) by phosphomolybdenum method

Antioxidant activity of the samples by phosphomolybdenum method was determined according to the methodology suggested by Prieto et al. [18]. In this context, a test solution was freshly prepared by combining each solution (0.6 M sulphuric acid (30 mL), 28 mM sodium phosphate (28 mL) and 4 mM ammonium molybdate 40 mL). Then, 0.4 mL of extract was mixed with 4 mL of test solution. After the test tubes were vortexed, they were incubated in a water bath at 95°C for 90 min. At the end of the period, the absorbance values of the samples were measured at 695 nm with a UV-vis spectrophotometer (UV-vis 1800, Shimadzu, Japan). Antioxidant activity results were given in mg ascorbic acid equivalent (mg AAE/kg) using the calibration curve prepared with the ascorbic acid standard.

2.5. Statistical analysis

In order to perform the optimization of the processing variables, Design Expert 7.0 software was used and the optimum pH and salt concentration, which provides maximum protein content of the final concentrate, was determined by the desirability function and determined as a result of validation processes. Besides, principal component analysis (PCA) and correlation analysis were performed to detect the relationship among genotypes in terms of bioactive and technological properties using PAST software.

3. Results And Discussions

3.1. Optimization of the protein extraction conditions

At the beginning of the protein extraction from the common bean genotypes, an optimization study was performed to determine the effects of alkali level (pH) and salt concentration on the protein content of the final concentrate using central composite rotatable design. Table 1 shows the levels of processing parameters and the protein levels of the concentrates at these processing conditions. As is seen, the protein content of the concentrate samples ranged between 62-79.03%. The lowest protein content was observed for the common bean sample exposed to extraction at pH 11 and no salt addition while the highest protein content was determined for the sample extracted at pH 11 and with the addition of 0.4% salt. It was observed that that the protein content was affected by the processing variables. Table 2 shows the effect of pH and salt concentration on the protein content of the final protein concentrates. It was seen that the linear effect of salt concentration showed significant effect on the protein content of the concentrate ($p < 0.05$) and linear effect of pH had no effect ($p > 0.05$). But the interaction effect of both salt level and pH showed very significant effect on the final product ($p < 0.01$). Figure 2 illustrates the change in protein content of the samples as surface plot and it could be seen that the increase in salt centered increased the protein level while the alkali level showed a slightly change for the response parameter. The counter plot also shows the change in the protein level depending on the processing variables and the increase could be seen towards to the highest level of both salt and pH level. Eq. 1 shows the constructed regression model which could be used efficiently to estimate the protein level of the common bean protein concentrate. As is seen, coefficient of determination for the regression model was calculated as quite close to unity which shows the fitting ability of the model.

Table 2
ANOVA table showing the effect of processing variables on the protein content of the concentrates

	Sum of		Mean	F	P-value
Source	Squares	df	Square	Value	Prob > F
Model	167.57	5	33.51	52.11	0.0003
X_1 -Salt (%)	44.35	1	44.35	68.96	0.0004
X_2 -pH	0.13	1	0.13	0.21	0.6693
$X_1 X_2$	122.69	1	122.7	190.78	< 0.0001
X_1^2	0.39	1	0.39	0.61	0.4716
X_2^2	0.01	1	0.01	0.01	0.9153
Residual	3.22	5	0.64		
Lack of Fit	2.67	3	0.89	3.29	0.2418
Pure Error	0.54	2	0.27		
Cor Total	170.78	10			
CV (%)	1.14				
R ²	0.962				
Adj R ²	0.981				

$$\text{Protein Content (\%)} = 70.24 + 2.72X_1 - 0.15X_2 + 5.54X_1X_2 + 0.39X_1^2 - 0.06X_2^2 \quad (R^2 = 0.982) \quad (\text{Eq. 1})$$

To reveal the best processing conditions for the protein extraction from the common beans, an optimization study based on desirability function was performed. Maximization and minimization process was applied and the minimum and maximum protein levels were calculated for the processing variables. Figure 3 illustrated the levels of processing variable concluding the maximum and minimum protein content. It was resulted that the maximum protein content would be at pH 11 and %0.4 salt concentration while the minimum protein level would be at pH 11 and 0% salt level. As could be seen from the figure, desirability function showing the accuracy of optimization results was calculated as 0.980 and 0.990 for the maximum and minimum values, respectively. Based on the optimization results, protein extraction from the different genotypes of common beans were performed at pH 11 and 0.4% salt concentrations. Similar pH value (pH 11) for the alkali extraction of protein from faba bean seed was used by Samaei et al. [19]. Karaca et al. [20] investigated the technological properties of protein extracted from different pulses and used salt extraction method due to the globulins rich in the legume seeds. Roy et al. [21] reported that the globulins are soluble in salty solutions and approximately 70% of the proteins in legume seeds is composed by globulins. It was also reported that the isoelectric precipitation technique provided the precipitation of globulins [22] while the mixture of globulins and albumins could be precipitated by salt extraction approach [23].

2.6. Physicochemical and technological properties of protein concentrates

Table 3 shows the protein content of the common bean genotypes and protein concentrates and yield values. As is seen, the protein level of the common bean flour ranged between 22-26.93%. The highest protein content was determined for G8 and the lowest was for G7 (Table 3, Fig. 1). The similar results were also reported by other researchers who informed that the protein content of the common beans had 20–30% of protein level and the genotypes affected the protein content of the samples [24]. Protein content of the protein concentrates was in the range of 72.97–77.99% while the highest level was observed for G8 and the lowest one was for G7. The highest protein level of the concentrate was determined for the common bean sample having the highest protein content (G8) while the lowest level of protein of the concentrate was for the common bean having the lowest protein content (G7). In another research conducted by Karaca et al. [20], the protein content of the protein concentrates obtained from lentil and soybean by salt extraction method were reported to be 74.71 and 72.64%, respectively. Aragundade et al. [25] studied the effect of ionic strength and pH precipitation effect on extractability of protein and reported that the protein content of the broad bean protein concentrate was 71.6 which is similar to the result of the current study. Yield of protein concentrates was calculated according to the approach described by Makeri et al. [26] and the values for each common bean genotype were tabulated in Table 3. The yield values of the protein concentrates ranged between 32.79–50.70 %. Gundogan and Karaca [27] reported that the yield values for the local bean samples were in the range of 39–46%. Makeri et al. [26] reported the yield values of protein concentrates from winged and soybean were 50.82 and 57.12%, respectively.

Table 3
Protein content of common bean flour and their protein concentrates

Genotypes	Common Bean Flour (%)	Protein concentrate (%)	Yield (%)
G1	26.21 ± 0.45	77.70 ± 0.47	32.79
G2	26.64 ± 0.25	75.88 ± 0.75	49.34
G3	23.93 ± 0.36	76.20 ± 0.84	50.70
G4	26.78 ± 0.41	77.78 ± 0.95	39.66
G5	26.59 ± 0.15	77.11 ± 0.35	32.83
G6	22.39 ± 0.45	74.45 ± 0.45	42.27
G7	22.00 ± 0.15	72.97 ± 0.62	43.82
G8	26.93 ± 0.52	77.99 ± 0.61	32.98

Table 4 shows the color properties and functional features of the protein concentrates of common bean genotypes. Color properties were recorded as L, a, and b values and the differences among the samples were significant for all color parameters. The lowest L value which means the darkest protein concentrate was G7 while the highest L value was for the genotype coded as G3. Redness (a) values ranged between 0.40–8.15 and the yellowness (b) values were in the range of 11.03–15.53. The highest redness was for G8 and the highest yellowness was also recorded for G8. Wani et al. [28] reported that the color properties of the different cultivars were recorded as L, a and b as in the range of 81.16–83.25, 0.14–1.70 and 10.64–12.57, respectively. In another study conducted by Joshi et al. [29], L, a and b values of lentil protein concentrates were reported as in the range of 69.2–92.0, 6.9–16.3 and 15.7–24.8, respectively. The color of the protein concentrates is affected by the sample type and also the variation due to the genotypic differences and the pigment intense on the seed coat [28].

Table 4
Some physicochemical and technological properties of protein concentrates

Genotypes	L	a	b	Solubility	WHC	OHC	EC	ES
G1	55.65 ± 0.07	4.26 ± 0.08	15.41 ± 0.07	90 ± 2.7	3.45 ± 0.04	3.43 ± 0.18	68.64 ± 1.93	87.08 ± 0.59
G2	57.95 ± 0.04	4.72 ± 0.05	11.67 ± 0.04	58 ± 3.5	3.71 ± 0.30	4.95 ± 0.18	96.40 ± 1.64	95.26 ± 2.14
G3	68.16 ± 0.04	0.40 ± 0.08	11.03 ± 0.02	32 ± 2.4	4.07 ± 0.10	3.45 ± 0.01	67.05 ± 3.91	82.91 ± 1.54
G4	65.36 ± 0.01	2.79 ± 0.02	12.50 ± 0.02	84 ± 1.8	3.61 ± 0.04	4.22 ± 0.03	63.81 ± 1.56	81.14 ± 3.10
G5	54.26 ± 0.00	4.00 ± 0.05	14.16 ± 0.02	90 ± 4.1	3.38 ± 0.06	3.64 ± 0.17	59.27 ± 3.01	82.16 ± 3.05
G6	62.76 ± 0.01	0.23 ± 0.09	11.60 ± 0.02	84 ± 2.4	3.58 ± 0.06	3.52 ± 0.11	67.95 ± 1.81	85.00 ± 1.41
G7	27.14 ± 0.04	4.74 ± 0.10	13.63 ± 0.06	76 ± 2.6	3.55 ± 0.01	3.31 ± 0.16	67.17 ± 3.49	92.06 ± 5.08
G8	37.21 ± 0.05	8.15 ± 0.02	15.53 ± 0.05	94 ± 4.1	3.55 ± 0.07	3.31 ± 0.18	63.33 ± 0.00	78.79 ± 1.71
WHC: Water holding capacity, OHC: Oil holding capacity, EC: Emulsion capacity, ES: Emulsion stability								

Solubility of the protein concentrates was recorded at pH 7 and they were in the range of 32–94% and the highest soluble protein concentrates were obtained by common bean genotype coded as G8 while the genotype coded as G3 showed the weakest solubility character (Table 4). Among the samples, only two genotypes showed quite weak solubility compared to others. Karaca et al. [20] reported that the solubility of the protein changes significantly according to the protein isolation method and they informed the solubility of lentil protein and soy protein concentrates were 89.88 and 96.79% at neutral pH, respectively. Also, using salt for the extraction of the protein from the structure of the protein source such as a pulse flour could be caused a decrease in the solubility of the protein at neutral pH [20]. Kimura et al. [30] informed that the globulin type in the different pulses showed significant effect on the technological characteristics of the final protein concentrates and the distribution of hydrophilic and hydrophobic amino acid residues on the molecular surface as well as amino acid compositions are important determinants of solubility pH dependence. For instance, after hydrolysis by an enzymatic process, polar and non-polar amino acid groups, could be exposed on the surface of protein molecules and these polar amino acids may interact with water molecules easily through hydrogen bonds and electrostatic interactions, and these process could provide the increased protein solubility [28].

Water holding and oil holding capacity values of the protein concentrates ranged between 3.38–4.07 and 3.31–4.95 g/g, respectively. G3 showed the highest WHC while the highest OHC value was calculated for the genotype coded as G2 (Table 4). In another research, kidney bean protein concentrates showed much higher WHC and OHC compared to our results and they were reported that the WHC and OHC of the concentrates ranged between 5.34–5.83 and 5.82–6.92 g/g, respectively [28]. Withana-Gamage et al. [31] informed the WHC of the chickpea protein concentrate as in the range of 2.34–4.31 g/g and WHC for the mung bean concentrates were reported to be ranged between 1.28–2.78 g/g [32]. In general, WHC showed a wide variation depending on the source. Makeri et al. [26] stated that the dissociation temperature level and pH conditions could result the subunits to create the more water binding sites than oligomeric proteins. As compared to the results in the literature, WHC of the protein concentrates from the common bean genotypes are higher than

those of most of the protein concentrate resources. WHC of the protein concentrates could be have a technological function in some food formulations need to high level of water absorption capacity such as meat-based products or baked dough [28]. Wit [33] informed that the imbibing of the water has a critical role for the quality and the product yield in sausages, comminuted and pumped meats. OHC of the protein is also important quality character for some food types including oil incorporation in the formulation such as meat, doughnuts, sausages, soups, custards, baked products, dairy products where they can provide some desirable properties such as body, thickening, and viscosity [34].

Two important quality characteristics of protein concentrates namely emulsion capacity (EC) and emulsion stability (ES) were determined and the calculated values for all genotypes were tabulated in Table 4. As is seen, EC values of the protein concentrates ranged between 59.27–96.40% while the ES values were in the range of 78.79–95.26%. The best emulsion forming performance was observed for the sample coded as G2 while the sample coded as G5 showed the weakest capacity. Similarly, the best ES performance was also monitored for the sample of G2. Significant variations among the EC of the common bean protein concentrates were observed. Hojilla-Evangelista et al. [35] informed that the emulsion activity showed significant variation for the common bean genotypes and the emulsion activity values were in the range of 51.8–65.8 m²/g at neutral pH for the samples and also they showed that the pH had a critical role for the EC because these values ranged between 378.8–387.9 m²/g at pH 10. Sahni et al. [36] also reported that the EC of the alfa-alfa protein concentrates were 1.91, 39.16 and 60.41% at pH 4, 7 and 9, respectively. Mao and Hua [37] reported that the EC of walnut protein concentrate (WPI) was 50.01% and they also stated that the pH had a significant effect on the EC. In neutral pH, EC of the WPI was approximately 50% and increase in pH increased the EC of the samples. It was concluded that EC was pH dependent and alkaline pH has an improver effect on the EC of the protein concentrates.

2.7. Bioactive properties of protein concentrates

Table 5 shows the bioactive characteristics of the common bean flours and their protein concentrates. As is seen, the common bean genotypes showed differences for the studied parameters for both flour and protein concentrates. Total phenolic content (TPC) of the flour and concentrates were in the range of 578.9–1355.9 mg GAE/kg and 313.5–1219.1 mg GAE/kg, respectively. The highest TPC was determined in G8 among the flours and G7 among the concentrates while the lowest ones were determined in G4 and G3 for flour and concentrates, respectively. Piñuel et al. [38] characterized the protein concentrates from red bean and reported that the TPC of the samples ranged between 1356–5217 mg GAE/kg. Total flavonoid content (TFC) of the samples ranged between 352.5–1185 mg QE/kg and 392–1253 mg QE/kg for the flour and concentrates, respectively. The highest TFC of the flours was in the genotype coded as G8 and then G7 while the highest TFC for the protein concentrates was observed in same genotypes.

Table 5
Bioactive properties of common bean flour and their protein concentrates

<i>Common bean flours</i>						
Genotypes	TPC (mg GAE/kg)	TFC (mg QE/kg)	ARA _{DPPH} (%)	ARA _{ABTS} . ⁺ (μg TE/g)	FRAA (mg AAE/kg)	AA (mg AAE/kg)
G1	1218.1 ± 9.4	982.5 ± 118.7	24.54 ± 2.37	3.205 ± 0.08	1.72 ± 0.06	5410.6 ± 254.9
G2	1058.2 ± 65.4	762.5 ± 41.9	20.61 ± 1.03	3.482 ± 0.45	1.69 ± 0.03	5109.6 ± 161.2
G3	996.5 ± 31.0	680.0 ± 46.9	25.81 ± 1.11	3.677 ± 0.43	2.00 ± 0.03	4792.2 ± 155.8
G4	651.0 ± 39.1	352.5 ± 28.7	15.18 ± 0.44	2.616 ± 0.34	1.45 ± 0.02	4262.7 ± 187.4
G5	578.9 ± 19.2	395.0 ± 17.3	14.85 ± 0.13	1.869 ± 0.17	1.35 ± 0.02	4314.8 ± 375.9
G6	1223.5 ± 32.5	856.0 ± 38.3	36.32 ± 2.37	4.473 ± 0.46	2.28 ± 0.01	4726.3 ± 449.5
G7	1200.5 ± 45.2	1080.0 ± 45.5	31.01 ± 1.74	4.001 ± 0.36	2.09 ± 0.06	5482.5 ± 230.3
G8	1355.9 ± 15.5	1185.0 ± 48	34.31 ± 1.35	4.318 ± 0.17	2.16 ± 0.04	5065.8 ± 203.1
<i>Protein concentrates</i>						
G1	828.1 ± 53.2	679.0 ± 89.1	29.07 ± 1.78	1.898 ± 0.317	1.26 ± 0.02	2793 ± 305.5
G2	829.4 ± 48.2	497.0 ± 69.3	23.28 ± 2.01	3.009 ± 0.846	1.10 ± 0.05	2894.1 ± 179.8
G3	313.5 ± 63.5	392.0 ± 19.8	24.80 ± 0.20	1.690 ± 1.200	0.81 ± 0.05	2443.0 ± 268.1
G4	352.7 ± 26.8	616 ± 39.6	25.97 ± 0.88	1.331 ± 0.046	0.89 ± 0.04	2985.5 ± 153
G5	338.8 ± 68.2	903 ± 29.7	27.91 ± 2.36	0.823 ± 0.148	0.78 ± 0.01	2229.1 ± 96.3
G6	409.4 ± 21.4	462 ± 19.8	26.93 ± 0.82	1.260 ± 0.074	0.85 ± 0.01	3016.6 ± 287.3
G7	1219.1 ± 59.1	1036 ± 39.6	37.32 ± 1.48	3.612 ± 0.334	2.21 ± 0.02	3533.8 ± 248.3
G8	1196.4 ± 53.5	1253 ± 148.5	36.49 ± 1.16	3.624 ± 0.448	2.14 ± 0.05	3590.2 ± 171.3
TPC: Total phenolic content, TFC: Total flavonoid content, ARA _{DPPH} : DPPH radical scavenging activity, ARA _{ABTS} . ⁺ ABTS. ⁺ radical scavenging activity, FRAA: Ferric reducing antioxidant activity, AA: Antioxidant activity by phosphomolybdenum						

Antiradical activity of all samples was evaluated by using both DPPH and ABTS.⁺ radical substances. The results of the antiradical activities of the samples were tabulated in also Table 2. DPPH radical scavenging performance of the flour and concentrate samples were in the range of 14.85–34.31% and 23.28–37.32% (Table 5), respectively. For the flour samples, the weakest scavenging activity was determined for the samples of G4 and G5 while the lowest antiradical performance was for the sample of G2 for the concentrate samples. Similar trends were also observed for the ABTS.⁺ radical scavenging activities of the samples. As is seen, ABTS.⁺ radical scavenging performance of the flour and concentrates ranged between 1.869–4.473 µg TE/g sample and 0.823–3.624 µg TE/g sample, respectively. The prominent flour and concentrate for the highest and lowest radical scavenging activity of ABTS.⁺ were G6 and G8, and G5 and G5, respectively. Antioxidant activity of both flour and concentrate was characterized by two popular methods namely ferric reducing (FRAA) and phosphomolybdenum antioxidant activity (AA) approaches. Samples showed differences in terms of antioxidant activities and the strongest antioxidant performances by FRAA were seen in G6, G7 and G8 for the flours and G7 and G8 for the protein concentrate samples. By phosphomolybdenum approach, G1, G7 and G8 among the flours and G7 and G8 among the concentrates were the prominent genotypes (Table 5).

2.8. Classification and correlation analysis of samples

To determine the similarity and differences among the studied common bean genotypes, PCA was used and the obtained factor loadings for the samples was illustrated in Fig. 4. The parameters whose curves direct close to one another on the loading plot mean positively related, while those whose curves appear in opposite directions are negatively related. And also the distance between the locations of any genotype on the score plot explains degree of difference or similarity between them [39]. As is seen, G2 showed a significantly different characteristics in terms of EC, WHC and OHC values of protein concentrates. G7 and G8 were also located in same group compared to other genotypes and differed from the other genotypes in terms of bioactive properties. Besides solubility and WHC values of concentrates directed oppositely on plot and that situation was also proved in correlation matrix as a negative correlation (Table 6). Same situation was observed in the study of Stone et al. [40] for pea protein concentrate and WHC was negatively correlated with solubility ($r = -0.857$; $p < 0.01$). Other positive correlations were observed between ARA_{DPPH} and TPC, TFC; ARA_{ABTS} and TPC; AA and TPC, FRAA and ARA; FRAA and TPC, TFC, ARA; a values and EC, ES, ARA_{ABTS} ; L values and OHC; OHC and EC. Siddiq et al. [41] also observed the positive correlation between emulsion capacity and stability for bean flours. No significant correlation between solubility and emulsion stability or capacity was detected.

Table 6
Correlation matrix for the studied parameters of protein concentrates

	TPC	TFC	ARA DPPH	ARA ABTS. ⁺	FRAA	AA	PC	L	a	b	Solubility	WHC	OHC	EC	ES
TPC	1														
TFC	0.691	1													
ARA DPPH	0.783	0.890	1												
ARA ABTS. ⁺	0.933	0.546	0.644	1											
FRAA	0.947	0.800	0.918	0.889	1										
AA	0.801	0.554	0.733	0.792	0.851	1									
PC	-0.208	0.103	-0.210	-0.267	-0.236	-0.282	1								
L	-0.194	-0.145	-0.490	-0.086	-0.330	-0.329	0.251	1							
a	0.624	0.023	0.043	0.734	0.403	0.319	-0.261	0.363	1						
b	-0.108	0.114	0.138	-0.287	-0.039	-0.356	0.062	-0.083	-0.254	1					
Solubility	0.269	0.603	0.478	-0.027	0.294	0.336	0.267	-0.107	-0.320	0.090	1				
WHC	-0.273	-0.562	-0.411	0.043	-0.242	-0.178	-0.131	-0.056	0.163	-0.244	-0.932	1			
OHC	-0.179	-0.439	-0.641	-0.017	-0.375	-0.172	0.115	0.872	0.502	-0.359	-0.242	0.141	1		
EC	0.200	-0.411	-0.427	0.358	-0.068	0.042	-0.202	0.520	0.853	-0.466	-0.416	0.275	0.781	1	
ES	0.357	-0.243	-0.110	0.397	0.144	0.122	-0.610	0.292	0.811	-0.023	-0.296	0.039	0.463	0.784	1
TPC: Total phenolic content, TFC: Total flavonoid content, ARA_{DPPH} : DPPH radical scavenging activity, ARA_{ABTS} : ABTS. ⁺ radical scavenging activity, FRAA: Ferric reducing antioxidant activity, AA: Antioxidant activity by phosphomolybdenum, PC: Protein content, WHC: Water holding capacity, OHC: Oil holding capacity, EC: Emulsion capacity, ES: Emulsion stability															
Values in bold with a significance level $\alpha = 0,05$															

In summary, firstly protein isolation conditions (salt and pH) were optimized for common bean and then genotypic variation for the important techno-functional and bioactive attributes of bean protein concentrates were identified. These differences should be further explored for more prominent genotype

selection in food industry applications.

Declarations

Acknowledgement

This study was financially supported by Erciyes University Scientific Research Projects Coordination Unit under Grant number of FBA-2018-7908.

Author contributions statement

B.C. conceived the experiments, K.K. and M.K. supervised the study, conducted the experimental design and optimization, and edited the manuscript. All authors reviewed the manuscript.

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Figures

Figure 1

Different common bean genotypes used for the production of protein concentrates

Figure 2

Effect of pH and salt level on protein content of concentrate produced from common beans

Figure 3

Maximization (a) and minimization (b) process for the protein content of the common bean

Figure 4

Factor loadings of the parameters for the protein concentrates of common bean genotypes