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CHEMICAL AND PHYSICAL CHARACTERIZATION OF COMMON BEAN (*PHASEOLUS VULGARIS L.*) LANDRACES BY NORTH – NORTH-WESTERN EXTREMITY OF ROMANIA

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Abstract: *The aim of our study was to characterize beans (Phaseolus vulgaris L.), one of the most important legumes at the international level, by examining their physical and biochemical properties, to highlight the importance of preserving local bean varieties in Romania, beans which are kept in the collection of the Mihai Cristea Suceava Plant Genetic Resources Bank. Local cultures of Phaseolus vulgaris, have been best preserved, especially in the Maramureş area, followed by Suceava area. Based on all 28 samples from the common bean germplasm collection, the mean values for seed size characteristics were seed length (L) 14.64 ± 2.24 mm and seed width (W) 8.93 ± 1.51 mm. The average weight of the 1,000-seed characteristic was 521.34 g, with the minimum and maximum values ranging from 136.96 to 1,045 g for all 28 samples. The highest coefficient of variation was calculated for 1,000-seeds weight (39.9 %) and the lowest for L/W (13.2 %). The protein content determined for 16 samples from the common bean germplasm collection was 23.79 ± 2.49 g/ 100 g of dry matter. The amount of protein varies between 18.84 g/ 100 g of dry matter (sample F23) and 26.69 g/ 100 g of dry matter (sample F27). The free amino acid content varies between 0.56 g and 1.29 g/100 g of dry matter, and the boiling time between 35 and 80 minutes. Boiling time is dictated by the variety of beans, but a very interesting thing, observed from the analyses carried out, is that the boiling time varies inversely with the percentage of protein. So, in sample F27 we have a boiling time of 35 minutes and a protein content of 26.69 g/ 100 g of dry matter. The sample with the highest protein content has the lowest boiling time. At the same time, sample F19 has a boiling time of 80 minutes and a protein content of 19.44 g/ 100 g of dry matter.*

Keywords*: the cooking time, free amino acids, local populations, quantitative seed descriptors*.

1. Introduction

The total disappearance of many plant species is one of the many consequences of anthropogenic activities, some of which have devastating environmental effects. Throughout its existence, humanity has used some 10,000 species of crop plants, but according to the latest reports from the Food and Agriculture Organisation of the United Nations (FAO) [1], 90% of food production is currently based on 120 species. The advent of industrialised agriculture has led to a drastic reduction in biodiversity and a marked process of genetic erosion. Modern varieties have been developed at the expense of the old and local varieties of crops [2]. At the national level, the most critical situation is observed in flax and hemp (fibre) growing, where local varieties have almost disappeared.
Phaseolus vulgaris crops are among the best preserved, especially in the Maramures area, followed by Suceava county. To preserve the agricultural genetic heritage on farms in Romania, specific governmental and political measures are needed, as well as the elaboration of a National

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Conservation Plan, involving farmers and small agricultural producers [3, 4].
Local varieties, which are in danger of extinction, are an invaluable source of plant genetic resources and are used to generate new varieties and hybrids with higher productivity than local varieties, resulting in sustainable agriculture and food security [5].
The FAO considers the common bean, or *Phaseolus vulgaris L.*, the most important vegetable in the world [1]. This particular importance for human nutrition is due to its chemical composition and also to its use as green pods, green and dried beans, bean meal, leaves, husks, and straw as animal feed. The chemical composition is mainly influenced by the state of growth of the bean (green, dry) and its variety; other factors that may influence it are climatic and soil conditions [3, 6].
Carbohydrates are found in the highest concentration in dried beans, ranging from 50 to 60 g/ 100 g of the dry matter [7, 8]. Among the polysaccharides, starch is the most abundant molecule. With 23-33% protein in dry matter, beans are an excellent source of protein and play an important role in human nutrition. Protein is rich in essential amino acids such as lysine, arginine, and tryptophan [8, 9]. The fat content is low, about 2g/100 g of dry matter. Most lipids are phospholipids. Beans are an important source of unsaturated fatty acids. Linoleic acid is the predominant unsaturated fatty acid in beans [8, 10]. Beans are an important source of minerals and vitamins, far exceeding the amounts provided by grains [8, 11].

Area of distribution of the genus *Phaseolus vulgaris* and cultivated area

Beans are grown all over the world where the soil and climate conditions are suitable for the plant's growth and development. Summer temperatures should be above 15ºC and minimum rainfall 150 mm, up to 45 - 500 northern latitudes. In the 2,000s, the main bean producers were the United States, China, France, Russia and Senegal [12].
In our country, between 1975 and 1977, beans were grown on an area of 83,700 ha, with a production per hectare of 600 kg [13, 14]. The last national statistics for bean production were made in 2017, with a total production of 16,125 tonnes and an average production of 1,402 kg/ha, with the highest production in the north-eastern part of Romania [15].
The aim of our study was to determine the physical and biochemical characteristics of local populations of *Phaseolus vulgaris*, maintained in the collection of the Mihai Cristea Suceava Plant Genetic Bank, in order to raise awareness of the importance of preserving local bean varieties in Romania, as a valuable source of qualitative and quantitative nutrients reaching the consumer.

2. Materials and methods

Plant Material

Twenty-eight common bean accessions, of which fourteen white common bean accessions, seven black common bean accessions and seven reddish-brown common bean accessions, (Figure 1), were made available for the study by the Plant Genetic Resources Bank "Mihai Cristea" Suceava [3]. Thus, the Plant Genetic Resources Bank of Suceava is responsible for the ex-situ conservation of plant resources.

Figure 1. Photo examples for common bean accessions (*Phaseolus vulgaris L.*) groups distribution according to seed color

Table 1.
Samples, sample registration number, locality (village, county) where collected, year of collection, species and colour

Sample name	Nr. acces	Locality	*Year*	*Varieties*	Colour
F1	SVGB-5794	Botiza, MM	1991	*Var. compressus*	White bean
F2	SVGB-1988	Bosanci, SV	1988	*Var. nanus*	White bean
F3	SVGB-13339	Vama, SV	2000	*Var. compressus*	White bean
F4	SVGB-14022	Cupșeni, MM	2001	*Var. ellipticus*	White bean
F5	SVGB-13753	Călinești, MM	2001	*Var. vulgaris*	White bean
F6	SVGB-13954	Lăpuș, MM	2001	*Var. vulgaris*	White bean
F7	SVGB-14128	Cernești, MM	2001	*Var. vulgaris*	White bean
F8	SVGB-14150	Tg. Lăpuș, MM	2001	*Var. vulgaris*	White bean
F9	SVGB-13977	Suciu de Jos, MM	2001	*Var. compressus*	White bean
F10	SVGB-14092	Dealu Mare, MM	2001	*Var. compressus*	White bean
F11	SVGB-13936	Mocira, MM	2001	*Var. compressus*	White bean
F12	SVGB-5714	Botiza, MM	1991	*Var. coccineus*	White bean
F13	SVGB-14046	Peteritea, MM	2001	*Var. compressus*	White bean
F14	SVGB-5791	Botiza, MM	1991	*Var. cocineus*	White bean
F15	SVGB-13775	Săpânța, MM	2001	*Var. vulgaris*	Reddish-brown bean
F16	SVGB-15959	Bistra, MM	2003	*Var. vulgaris*	Reddish-brown bean
F17	SVGB-13752	Călinești, MM	2001	*Var. vulgaris*	Reddish-brown bean
F18	SVGB-13685	Lunca de la Tisa, MM	2001	*Var. vulgaris*	Reddish-brown bean
F19	SVGB-13674	Bistra, MM	2001	*Var. vulgaris*	Reddish-brown bean
F20	SVGB-13778	Sarasău, MM	2001	*Var. vulgaris*	Reddish-brown bean
F21	SVGB-15919	Plopișor, MM	2003	*Var. vulgaris*	Reddish-brown bean
F22	SVGB-15964	Ieud, MM	2003	*Var. vulgaris*	Black bean
F23	SVGB-14003	Groșii Țibleșului, MM	2001	*Var. vulgaris*	Black bean
F24	SVGB-8759	Cupșeni, MM	1993	*Var. vulgaris*	Black bean
F25	SVGB-10423	Moisei, MM	1994	*Var. vulgaris*	Black bean
F26	SVGB-13639	Repedea, MM	2001	*Var. ellipticus*	Black bean
F27	SVGB-13687	Lunca de la Tisa, MM	2001	*Var. vulgaris*	Black bean
F28	SVGB-13777	Săpânța, MM	2001	*Var. vulgaris*	Black bean

Legend: *MM – Maramures county, SV – Suceava county*

At present, the bank's collection contains about 18,000 unique varieties at $+4^0$C and -20^0C respectively. Common bean accessions were collected from 21 villages in two counties - Maramureș and Suceava, (Table 1, Figure 2), - of the North - North-

West region of Romania, from people's gardens, representing local populations, seventeen of the species *Phaseolus* var. *Vulgaris*, two common bean accessions var. *Coccineus*, six common bean accessions var. *Compressus*, one common bean accessions var. *Nanus*, two common bean accessions var. *Ellipticus*. Samples were collected in different years and analyses were performed on the original sample, preserved at +4^{0}C in Table 1.

Fig. 2. Map of the North - North-West region of Romania presenting the common bean sampling villages (counties - Maramures and Suceava)

The sampling area of a common bean accession is located in the extreme north-north-western part of Romania, at the border with Hungary (N-W) and Ukraine (N) and covers the administrative territory of Maramures-MM and Suceava - SV counties. The climate of the area is temperate continental, with oceanic influences in the west and continental influences in the east. These influences are reflected in seasonal temperatures (January - February, T_{min} = -30.0^{0}C; July - August, T_{max} = 39.0^{0}C) and rainfall (February - March, P_{min} = 32.6 mm; May - June P_{max} = 205.4 mm).

Seed evaluation using quantitative characteristics

Quantitative seed descriptors included the evaluation of the following five characteristics: seed length (L) [mm]; seed width (W) [mm]; seed length/width ratio (L/W); 1,000 (for common bean) seed weight [g] [16].

Sample preparation

Broken and damaged beans, as well as foreign materials, were removed.

Then, the samples were ground in a mill to obtain a fine and homogeneous powder using an IKA A11 Analytical Mill (Sigma-Aldrich, Germany), and maintained at -18°C until analysis.

Protein quantity

Protein content was determined using the micro-Kjeldahl method and a conversion factor of 6.25 [17, 18]. The total amino acids were verified the method based on the interaction of the primary amine group present in amino acids with ninhydrin. Reaction product of ninhydrin with primary amino groups produces the coloured chromophore called as Rheumann’s purple [19]. The absorbance was read at 530 nm with a T70+ UV/VIS Spectrometer (PG Instruments Ltd, United Kingdom), using deionized water as the blank and 0.5 cm optical path. The and free amino acids were expressed as amino nitrogen [%], using a calibration curve in the range of 0.1...0.25 mg tyrosine/ml of a freshly prepared tyrosine solution. The results will be expressed in percentages for the analysed product and are calculated using equation 1:

$$\text{amino nitrogen } \% = \frac{G \cdot d}{m \cdot 100} \cdot 100 \qquad (1)$$

where,
G = the amount of amino nitrogen or tyrosine determined from the calibration curve, [mg];
m = the quantity of product taken into analysis, [mg];
d = the dilution used.

The cooking time

To determine the cooking time, the beans were placed in water at 100°C and the time elapsed from that moment until the texture of the bean softened was timed. Bean texture was analysed subjectively by sticking a needle into the bean.

3. Results and discussion

Seed Characterization of Common Bean Accessions (*Phaseolus vulgaris L.*)

A total of four seed morphological characteristics, i.e., quantitative (seed length - L; seed width - W; seed length/width ratio - L/W and 1,000 seed weight) were evaluated. In the upper part of Table 3 are presented summary statistics for four quantitative common bean seed characteristics. The mean values for seed size characteristics based on all 28 accessions in common bean germplasm collection were for L 14.64 ± 2.24 mm, and for W 8.93 ± 1.51 mm (see Table 3). The minimum and maximum values ranged between 10.01 and 19.5 mm for L, and 6.07 and 12.95 mm for W. For characteristic1,000 seed weight the mean value of all 28 accessions was 521.34 ± 208.19 g, while the minimum and maximum values ranged between 136.96 and 1,045 g. The highest coefficient of variation was calculated for the 1,000 seed weight (39.9%) and the lowest for L/W (13.2%). As discussed by Rana *et al.* [20] for 4,274 common bean accessions conserved in Indian gene bank, L ranged 5.0 – 20.3 mm, W 2.0 – 12.0 mm and 100 seed weight 3.5 – 96.3 g. Similarly, Kara *et al*. [21] reported for 12 registered Turkish common bean accessions genotypes L 9.1 – 17.8 mm, W 5.8 – 10.0 mm, T (seed thickness) 4.6 – 6.0 mm and 100 seed weight 18.0 – 65.6 g. As discussed by Giurcă [22] for 9 common beans accessions originating from northern Romania and western Ukraine, L ranged 11.8 – 18.0 mm, W 7.4 – 9.7 mm, T (seed thickness) 4.4 – 6.9 mm and 100 seed weight 34.3 – 54.2 g. Logozzo *et al.* [23] evaluated 533 accessions of the European common bean germplasm and reported accessions with L 12.0 – 13.9 mm (35.5%), W 7.1 – 8.0 mm (33.0%) and T 5.0 – 5.9 mm (37.1%) were the most frequent. As discussed by Sinkovič *et al*. [16] for 953 common bean accessions conserved in Slovene gene bank at the Agricultural Institute of Slovenia, being part of the National plant gene bank, L (range 7.3 – 27.2 mm); T (range 4.2 – 11.0 mm); W (range 0.3 – 16.5 mm); L/W (range 0.4 – 2.6 mm); W/T (range 0.6 – 2.2 mm); and 100 for common (range 19.3 – 98.4 mm) bean seed weight.

Table 3.
Summary statistics for four quantitative seed characteristics in accessions of common (*Phaseolus vulgaris L.*)

Characteristics	Range	Mean ± SD	CV (%)
seed length (L) [mm]	10.01 – 19.5	14.64 ± 2.24	15.3
seed width (W) [mm]	6.7 – 12.95	8.93 ± 1.51	16.9
seed length/width ratio (L/W)	1.2973 – 2.1847	1.66 ± 0.22	13.2
1,000 seed weight [g]	136.96 – 1,045	521.34 ± 208.19	39.9

All seed characteristics measured quantitatively showed wide range of variation among all common bean accessions evaluated. Frequency distribution graphs of 28 common bean accessions for quantitative seed characteristics are shown in Figure 3.

Based on quantitative measurements, the common bean accessions were classified according to the L into two groups, i.e., medium, and large.

The first group included accessions with medium seeds measuring L from 10.0 to 15.0 mm (16 accessions or 57.14 %);
and the second group accessions with large seeds and L > 15.0 mm (12 accessions or 42.86 %), (Figure 3a). From the point of view of the width of the accessions, the samples are divided into two broad and narrow groups. The first group included broad-seeded accessions measuring W from 8.925 to 12.95 mm (11 accessions or 39%); and the second group of accessions with narrow seeds W < 8,925 mm (17 accessions or 60.71 %), (Figure 3b). Similarly, the common bean accessions were classified according to the 1,000 seed weight into three groups, i.e., low-weight, medium-weight and high-weight.

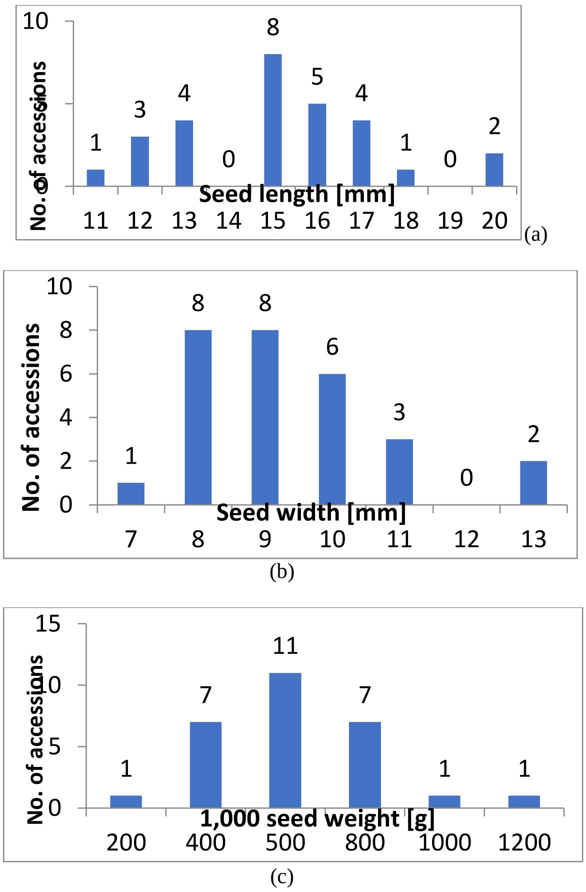

Fig. 3. Frequency distribution of 28 common bean accessions (*Phaseolus vulgaris L.*) for quantitative seed characteristics ((a) - seed length; (b) - seed width; (c) – 1,000 seed weight)

Low-weight seeds group included common bean accessions with 1,000 seed weight < 500.0 g (19 accessions or 67.85 %); the medium-weight seeds group accessions with 1,000 seed weight measuring from 500.0 to 1,000.0 g (8 accessions or 28.57 %); and the high-weight seeds group accessions with 1,000 seed weight >1,000 g (1 accessions or 3.57 %), (Figure 3c).

Protein quantity

Beans are rich in protein (21 – 25 %) [18, 24], carbohydrate (56 %), dietary fibre, and are a good source of antioxidants [25], as well as vitamins (vitamin B_1 (0.5 mg %), vitamin B_2 (0.4 mg %), niacin (3.4 mg %), vitamin C (20 mg %)) and minerals (potassium (1,770 mg %), calcium (195 mg %), phosphor (420 mg %), iron (7 mg %), nickel, cuprum, cobalt) [8, 11, 24, 26].
Unlike cereal proteins, the proteins present in beans accessions are considered to be the range of meat protein, with a range of 20 – 30 % proteins. Beans are rich in globulins which constitute 50 – 70 % of the total proteins along with a considerable amount of glutelins [27] (20 – 30 %), prolamin (2 – 4 %) and free amino acids (5 – 9 %) [28]. A glycoprotein, phaseolin consisting of neutral sugars especially mannose is also found in beans accessions. Beans accessions are also rich source of essential amino acids including lysine which is deficient in cereals [29].
The amount of protein in fresh beans averages 23 g / 100 g of dry matter, as the authors mentioned above reported. According to the determination of the amount of protein in beans that have been kept in the Bank's collection since 2001, high percentages can be seen in Table 4 and in the graphical representation in Figure 4. The mean values for the amount of protein based on 16 accessions in the common bean germplasm collection were 23.79 ± 2.49 g/ 100 g of dry matter. The amount of protein ranged from 18.84 g/ 100 g of dry matter (sample F23) to 26.69 g/ 100 g of dry matter

(sample F27). Twelve samples (F1 – F3, F7, F12, F14, F16, F20, F21, F22, F24, F25) were not representative in terms of protein and amino acid content, respectively cooking time; and they were not represented graphically (Figure 4, Figure 5, Figure 6, Figure 7). The difference between the minimum and maximum values is quite large and can be explained by differences at the molecular level, in the genes that determine the amount of protein. Of course, the phenotypic and genotypic values of the quantitative trait are determined by the amount of improvement, the deviation due to dominance, the deviation due to the interaction of the non-allelic or epistatic genes and the environmental deviation of sample F23 from Groșii Țibleșului, MM and sample F27 from Lunca de la Tisa, MM.

Table 4.

Summary statistics for protein quality in accessions of common (*Phaseolus vulgaris L.*)

Characteristics	**Range**	**Mean ± SD**	**CV (%)**
protein content [g/100 g of dry matter]	18.84 – 26.69	23.79 ± 2.49	10.5
free amino acids [g/100 g of dry matter]	0.56 – 1.29	1.019 ± 0.21	20.8
the cooking time [min.]	35 - 80	52.37 ± 12.93	24.7

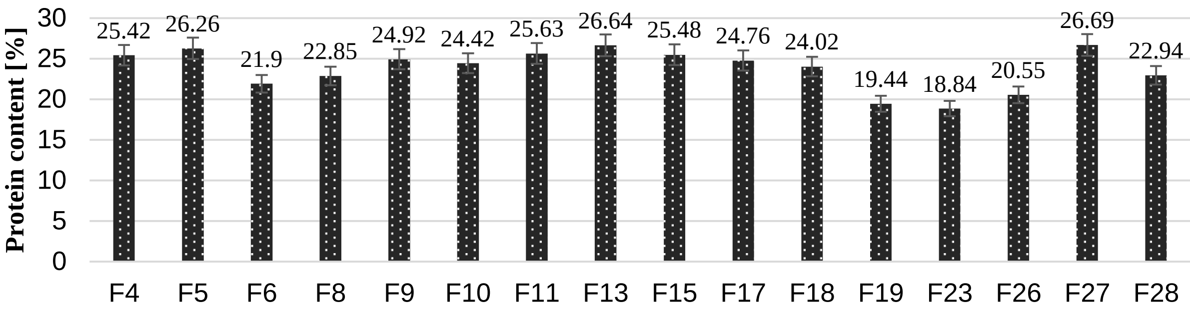

Fig. 4. The variation on protein content of 28 common bean accessions

Figure 5 shows the variation in protein content of the 16 common bean accessions (*Phaseolus vulgaris L.*) according to three varieties: var. *Compressus*, var. *Vulgaris* and var. *Ellipticus*. In the case of var. *Vulgaris* the variation in protein content is significant between 18.84 g/ 100 g of dry matter (sample F23) and 26.69g/ 100 g of dry matter (sample F27), while in var. *Compressus* is insignificant between 24.42 g/ 100 g of dry matter (sample F10) and 26.64 g/ 100 g of dry matter (sample F13).

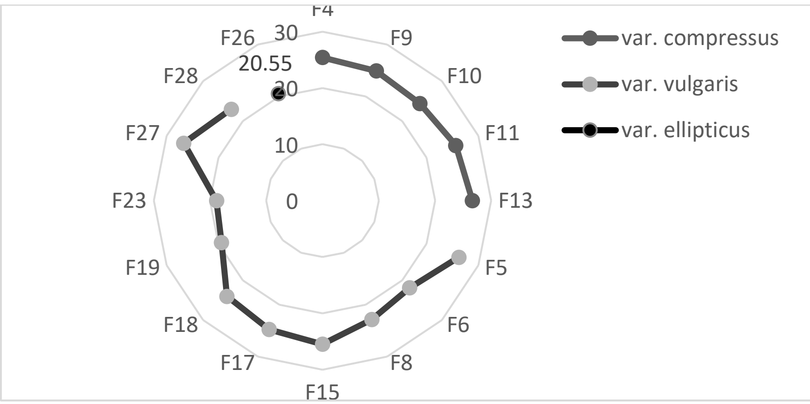

Fig. 5. The variation on protein content of common bean accessions (*Phaseolus vulgaris L.*)

The content, Figure 6, in free amino acids varies between 0.56 g/ 100 g of dry matter (sample F13) and 1.29 g/ 100 g of dry matter (sample F9), with an average of 1.019 ± 0.21 g/ 100 g of dry matter, and cooking time between 35 and 80 minutes, Figure 7. The cooking time is determined by the variety of beans, but a very interesting fact, observed in the analysis, is that the cooking time varies inversely with the percentage of protein.

So, in sample F27 we have a boiling time of 35 minutes and a protein content of 26.69 g/ 100 g of dry matter. The sample with the highest protein content has the lowest boiling time. At the same time, sample F19 has a boiling time of 80 minutes and a protein content of 19.44 g/ 100 g of dry matter.

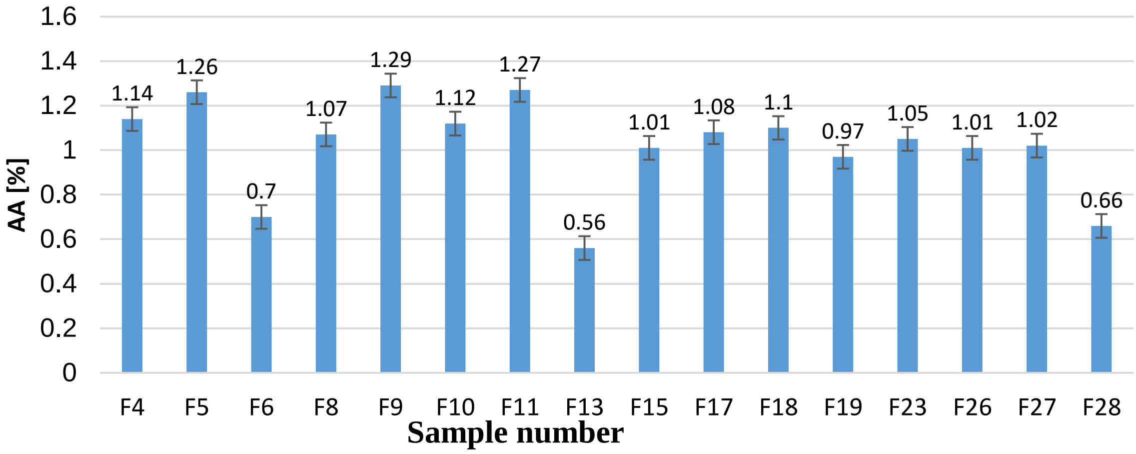

Fig. 6. The variation on free amino acid content of bean samples (*Phaseolus vulgaris L.*)

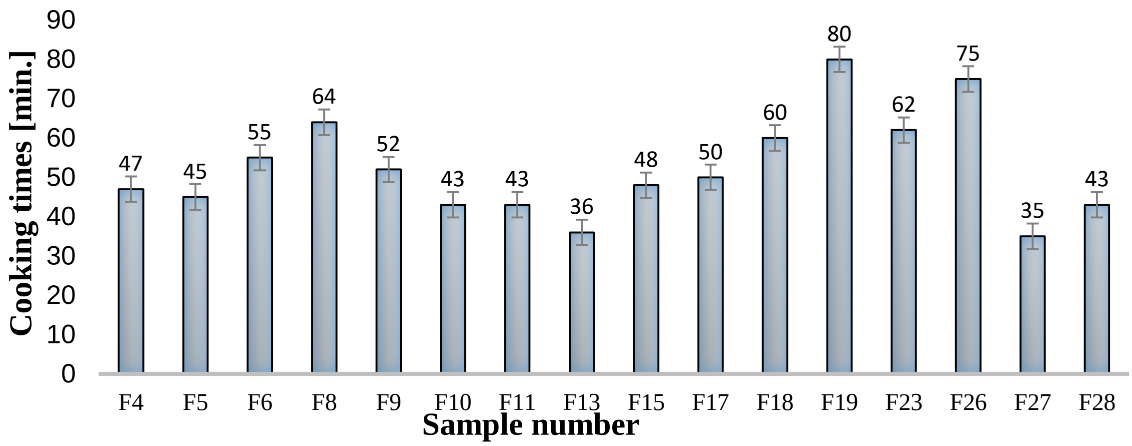

Fig. 7. The variation on the cooking time of common bean accessions (*Phaseolus vulgaris L.*)

Research over time has shown that boiling time is not influenced by specific genes, but more by storage conditions and storage time [30].

However, in a recently published study, Cichy K. A *et al*., 2015 [31] uses an experiment to show that specific genes can further affect boiling time. On the bean Pv06 chromosome, three genes encoding UDP-glucosyl transferases, a family of genes involved in flavonoid synthesis and pigment development, were found to be of great interest, as bean colour was correlated with the cooking time. Other candidate genes for bean cooking time, also located on chromosome Pv06, are AtCHX3 and

AtCHX4, which are related to ion exchange at the molecular level, H^+ and Ca^{2+}. There is evidence that the Ca^{2+} ion leads to an increase in cooking time. Akond *et al.*, 2011 [32] also show that white beans boil faster than coloured beans due to a lower content of phenolic compounds. The longest cooking time was found in the white cranberry bean [33].

In our case, sample F13 (36 minutes) boiled very quickly, being white, and sample F19 (80 minutes) boiled the hardest, being reddish-brown. But in our case we have an exception, sample F27, which boiled the fastest (35 minutes), being black in colour, (Figure 7).

4. Conclusion

Beans (*Phaseolus vulgaris L.*) are the most important legume internationally, being a valuable source of protein, starch, fibre, antioxidants, minerals and vitamins [34, 35, 36].

Following the determinations, we obtained the following:

- The mean values for seed size characteristics based on all 28 accessions in common bean germplasm collection were for L 14.64 ± 2.24 mm and for W 8.93 ± 1.51 mm.
- Protein content ranged from 18.84 - 26.69 g/ 100 g of dry matter.
- Total amino acid content ranged from 0.56 - 1.29 g/ 100 g of dry matter.
- The sample with the highest protein content has the lowest boiling time.

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