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Crude Protein and Ash Content in Different Coloured *Phaseolus coccineus* L.

Liene Strauta, Sandra Muizniece-Brasava, and Ina Alsina

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Abstract*—Phaseolus coccineus* L. is the third most important cultivated Phaseolus species in the world. It is widely grown in Latvia due to its earliness, good taste and uniform and qualitative yield. Experiments were carried out in the laboratories of Department of Food Technology and Agronomical Analysis Scientific Laboratory at Latvia Universityof Agriculture. Beans (*Phaseolus coccineus* L.) crude protein, crude ash content as well as colour measurements were analyzed. Results show, that brown coloured beans have less crude protein content than others, and ash content have significant differences.

Keywords*—Phaseoluscoccineus*, protein, ash, colour.

I. Introduction

LEGUMES occupy an important place in human nutrition, especially in the dietary pattern of low-income groups of people in developing countries. But even if legumes are considered as poor man's meat, they are generally good sources of nutrients [1]. They are an important and inexpensive source of protein, dietary fiber and starch for a large part of the world's population, mainly in developing countries and vegetarians to balance their diet [2], [3].

Beans (*Phaseolus*) is the most widely produced and consumed food legume in world, most in Africa, India, and Latin America [4]. They usually contain 20-30% protein on a dry basis [5], sometimes less. The storage proteins beans have recently attracted much attention, due to their superior functionalities, e.g., protein solubility, emulsifying properties and ability to form heatinduced gels [6], [7].

Common bean (*Phaseolus vulgaris* L.) is low in fat and rich in proteins, vitamins, complex carbohydrates, and minerals. In addition to contributing nutritional requirements, consumption of dry beans has been linked to reduced risk of heart disease [8], obesity [9] and cancer [10], [11]. Economically, beans are an important crop not only in North America, since their production and export has increased significantly in recent years, but in Canada, navy and pinto beans are among the most produced and consumed pulses [12]. However, widespread use of beans as a primary staple food has been limited by the presence of anti-nutritional factors, which might produce adverse effects for human and animal nutrition. Some of these compounds include enzyme inhibitors, lectins, phytates, cyanoglycosides, and phenolics [13]. Some data on *Phaseolus vulgaris* have focused on antinutritional aspects of seed coat polyphenols [14], [15]. However, polyphenols have contradicting positive effects on human health and it has been reported that they have anticarcinogenic and antioxidant properties [16]. It is generally believed that antioxidants scavenge free radicals and reactive oxygen species and can be extremely important in inhibiting oxidative mechanisms that lead to degenerative diseases [17]. More over recently, antioxidant activity was reported in extracts, condensed tannins, and pure flavonoids from coloured genotypes of common bean seed coats [17]–[19].

Because of their nutritional and health-promoting properties, the development of value-added bean-based products for new market opportunities in the functional food and nutraceutical industry is being promoted [20]. In this context, even the use of isolated bean hulls as an ingredient for novel food products featuring high dietary fiber and high antioxidant levels appears promising, in particular, for the ready-to-eat and snack food markets have been suggested [12].

Colour of seed testa is important for the marketing of faba bean for human consumption. Across different faba bean varieties, seed testa colour ranges from white to purple but the preferred colour has variously been described as beige, light tan or buff [21]. Light brown or beige is also the most common (91% of accessions at ICARDA) seed coat colour in faba bean at harvest [22]; however it is not stable and darkens during storage. Seed coat colour may change to medium brown, dark brown and even chocolate brown depending upon the storage conditions and duration. Postharvest colour darkening of faba bean reduces its value and market opportunity. Consumers and processors are reluctant to purchase darkened seed because colour is considered as an index of quality or freshness and consumers associate dark colour with old seed [23]. Furthermore, during heat processing or canning the immersion liquid or broth changes to a dark muddy colour [24]. Thus dark seeds are unacceptable to the unprocessed as well as the canning market. Storage conditions strongly influence the stability of postharvest seed colour in many types of beans. In other legumes there is some evidence that temperature, relative humidity (RH), seed moisture content (SMC) and light are the main factors that affect the stability of seed colour during storage [23]–[27]. High temperature (24°C) and high RH (80%) accelerated darkening in kidney beans (*Phaseolus vulgaris* L.) while beans stored at low temperature (1°C) and RH (30%) retained their original colour for one year [23]. Storage of chickpea (*Cicer arietinum* L.) at 33–35°C and 75% relative humidity for 160 days caused postharvest test colour darkening which was reflected bydecrease in Hunter 'L' value and increase in total colour difference (delta E) [28]. Lentil (*Lens culinaris* Medic.) seeds

L. Strauta, S. Muizniece–Brasava, and I. Alsina are with the Faculty of Food Technology, Latvia University of Agriculture, Jelgava, Latvia, LV–3001 (e–mail: kunka@inbox.lv, sandra.muizniece@llu.lv, ina.alsina@llu.lv).

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exposed to moderately high temperature (20 and 30°C) at high RH (100%) turned brown in 3 weeks or less while at cool temperature (5°C) with same RH (100%) browning did not occur before 5 weeks [26], [29]. Similarly little change in postharvest seed coat colour occurred in Rwandan dry beans (*P. vulgaris*) stored at 4°C for 24 months [30]. Light-red kidney beans also retained their original colour for one year when stored at 1°C [31]. Even at moderately low temperature (10°C) darkening was slow in adzuki beans (*Vigna angularis*) [32], [33].

Dry beans are rich in non-nutrient components; too [34] investigated the oxygen radical absorbance capacity (ORAC) of common foods consumed in the U.S. Their data showed that red kidney beans had the highest total antioxidant capacity per serving size as compared to all other foods, including many fruits commonly believed to be rich in antioxidants. The inclusion of legumes in the daily diet has many beneficial physiological effects in controlling and preventing various metabolic diseases such as diabetes mellitus, coronary heart disease and colon cancer [5]. It has been reported that the protective effects of dry beans in disease prevention, such as against cancer, may not be entirely associated to dietary fiber, but to phenolics and other non-nutritive compounds [35], as polyphenols from dry beans may possibly act as antioxidants, hindering the formation of free radicals [36]. In addition, legumes belong to the food group that elicits the lowest blood glucose response. The general consensus on healthy eating habits favours an increase in the proportion of legume-based polymeric plant carbohydrates including starch in the diet. The role of legumes as a therapeutic agent in the diets of persons suffering from metabolic disorders has been reported previously [37]. Dry bean flours can be used as functional ingredients to improve the nutritional quality of a variety of processed food products [38]. One recent study reported on the use of pinto bean flour in tortillas [39]. The application of various technological processes to legumes can increase their use as an ingredient in manufactured food products. Processing improves the nutritional quality of dry beans by reducing the content of anti-nutritional factors and, at the same time, diversifies their use as ingredients by altering their functional properties. The fact that dry beans, apart from being nutrient-rich, are gluten-free offers significant opportunities for exploiting bean flour use in different food systems [40].

Though *Phaseolus vulgaris* L. lately have been given more attention, *Phaseolus coccineus* L. are too widely used in daily human diet. So the aim to this research is to determinate if crude protein and ash content is influenced by *Phaseolus coccineus* L. colour.

II. Materials and Methods

A. Experimental Design

Experiments were carried out in the laboratories of Department of Food Technology and Agronomical Analysis Scientific Laboratory at Latvia University of Agriculture. Beans (*Phaseolus*) with different colours (Fig. 1) were used in this experiment. Beans were kept in room temperature and dried for month, but no other treatment as heating was made.

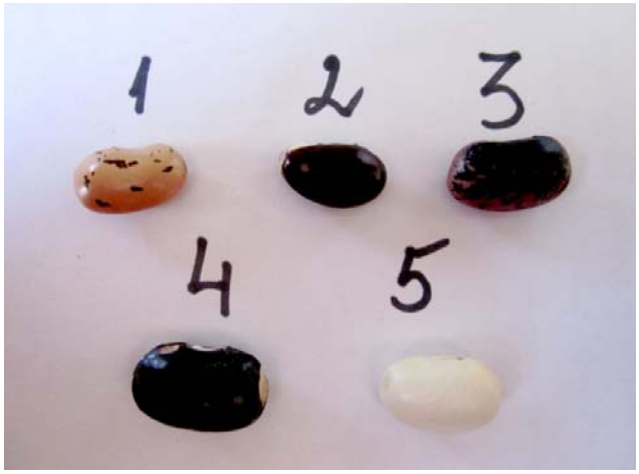


Fig. 1 Beans (*Phaseolus coccineus* L.) used in experiment 1-beige beans; 2-brown beans; 3-black/violet beans; 4-black beans; 5-white beans

B. The Physical Analysis

Colour of wheat bread was measured in CIE L*a*b* colour system using Tristimulus Colorimeter, measuring Hunter colour parameters by Colour Tec PCM/PSM. Colour values were recorded as L* (brightness) – the vertical co-ordinate runs from L* = 0 (black) through grey to L* = 100 (white); a* (-a, greenness, +a, redness) – the horizontal co-ordinate, that runs from -a* (green) through grey to +a* (red) and b* (-b, blueness, +b, yellowness) – another horizontal co-ordinate, that runs from -b* (blue) through grey to +b* (yellow) [41]. The measurements were repeated on different randomly selected locations at the surface of each sample.

C. The Chemical Analysis

The dry matter content was determinate according to ISO 6496, 1999. Container with lid for half an hour was dried at 103±3°C temperature, then cooled to room temperature in a desiccator. Weigh was measured with an accuracy of 1 mg. 5 g of sample weighed to the nearest 1mg, placed in container and put in the drying oven at 103±3°C for 4.0±0.1h. After four hours, the container lid were put on, sample was removed from oven and cooled in a desiccators to room temperature. Sample was weighted with container and absolute dry matter was calculated (ISO 6496, 1999). Absolute dry matter was used in the calculations of other parameters.

Crude protein (CP) determination by Kjeldahl method

Well crushed sample was weighted approximately 0.5g (to the nearest 0.0001g). The sample was transferred in temperature resistant glass flask, then copper catalyst was added as well 20mL of concentrated H_2SO_4. Flask was placed in a preheated stove (420±5°C), gas vacuum suction cap was fixed on and wet mineralization went for 1 hour until the solution in flask was bright and clear. Flask and digested sample was removed from the stove and allowed to cool for 15−20min. Cooled sample was placed in distillation unit, 50

mL of water, 80mL of 40% NaOH were added. Ammonium was distilled in 65mL of 4% H_3BO_3 solution. The steam distillation was carried out for 220s, then boric acids solution was titrated with 0.2*M* HCl (which concentration was checked with 0.1*M* NaOH solution) till pH 4.70. As blank sample 1g of sucrose, prepared the same as samples was used. Coefficient was used 6.25 (LVS EN ISO 5983-2, 2009)

Ash content was determinate with ISO 5984:2002/Cor 1:2005, where 5g of sample is weighted to nearest 0.0001g and placed in the porcelain crucible. For 20 minutes placed on oven. After that sample is placed in muffle for 4 hours. After samples are placed in exsiccator and cooled till room temperature, then weighted.

D. Statistical Analysis

The results were processed by mathematical and statistical methods using MS Excel 2007 One way and two way ANOVA and SPSS 14.0 multiple regression and correlation analysis.

III. Results and Discussion

Colour is an important attribute because it is usually the first property the consumer observes. The Hunter (L*, a*, b*) values were measured in order to describe the colour of beans (Figs. 2–4). In order to track precise bean colour measurements of colour were made. Coordinates of beige beans (sample 1) are shown in Fig. 2.

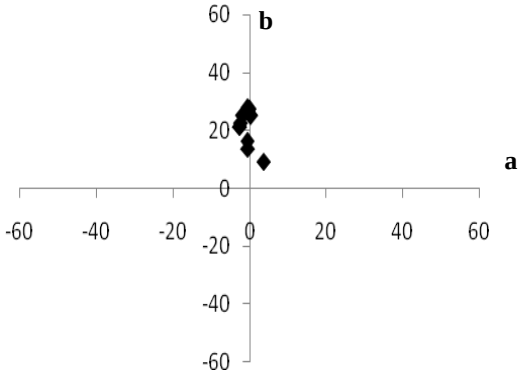


Fig. 2 Colour range of beige bean (sample 1). a* – ass as ordinate, and b* – as coordinate

Average L* coordinate is 64.02±3.09 (Fig. 7) showing that beans are lightly coloured, but a* average coordinate is -0.4±0.7 and b* 25±2. The points are gathering on b* axle, higher than 0, indication yellow colour domination in bean husk.

Average L* coordinate is 23.03 ±3.01 (Fig. 7), showing that beans are dark, but a* average coordinate is 7.03 ±1.00 and b* 10.01±8.00. As it is shown in Fig. 2 points are scattered around an axle, more in I quadrant (Fig. 3). As it can be seen, bean colour is not homogeneous, that is why some measurements in b* axle is below 0.

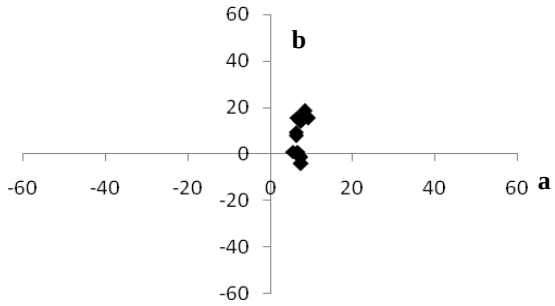


Fig. 3 Colour range of brown bean (sample 2). a* – ass as ordinate, and b* – as coordinate

And compared to first beans (sample 1), for these measurements are move more to a* axles positive measurements, indication more red colour in then, than in beige ones.

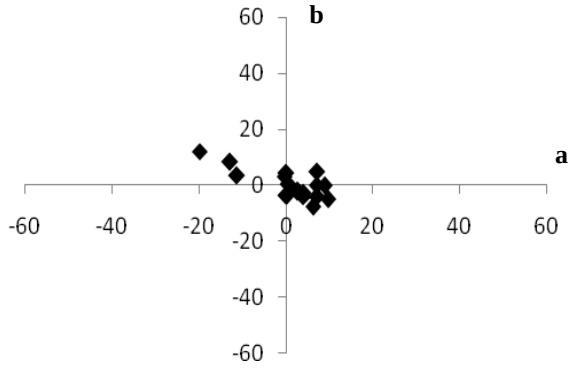


Fig. 4 Colour range of black/violet bean (sample 3). a* – ass as ordinate, and b* – as coordinate

Average L* coordinate is 27.00±6.01 (Fig. 7), but as beans are visibly two coloured, data was split in two groups, one representing black colour, with average L*=23.01±4.02, a*=-0.25±7.00 and b*= -0.02±4.00, and another representing violet colour with L*=35.01±1.00, a*=4.00±3.01 and b*=-0.12±3.00. Wide range of standard division can be explained with fact that colours are close, and in one measurement black and violet colours can overlap giving average result for whole bean (Fig. 4).

Averagely these beans can be described in colour coordinates L*=27.01±6.01 (Fig. 7), a*=0.90±8.00 and b*=-0.06±5.00.

As beans are two coloured it can be seen in measurements, as one group is placed more to red light and other more to green.

Fig. 5 shows black bean (sample 4) colour range with average coordinates L*=22.00±2. 01 (Fig. 7), a*=2.10±5.00 and b*= -5.01±11.02. It can be seen, that despite that with eye beans seem totally black, they are not, as shows L* value. Still they have some lighter colour.

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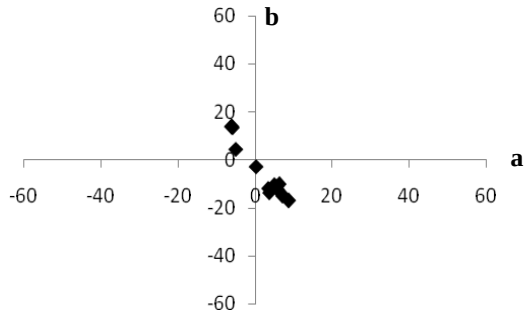


Fig. 5 Colour range of black bean (sample 4). a* – ass as ordinate, and b* – as coordinate

As measurements are moved more to III quadrant is indication that in these beans blue and red colour dominates over yellow and green.

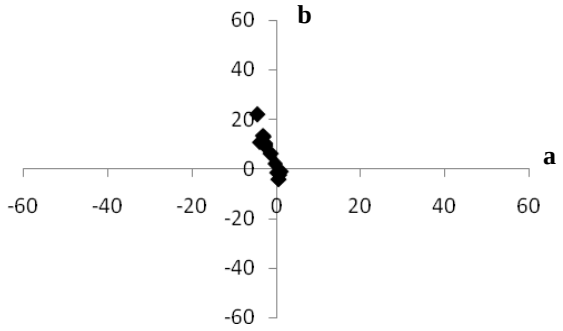


Fig. 6 Colour range of white bean (sample 5). a* – ass as ordinate, and b* – as coordinate

Fig. 6 shows white beans colour range with average coordinates L*=88.02±4.03 (Fig. 7), a*=-1.50±2.01 and b*=5.01±7.01. There are traces of yellow in these beans that could be caused by storage. All L values for beans are shown in Fig. 7.

Compared to black beans in this case measurements are move more to II quadrant and have more yellow colour, than previous beans.

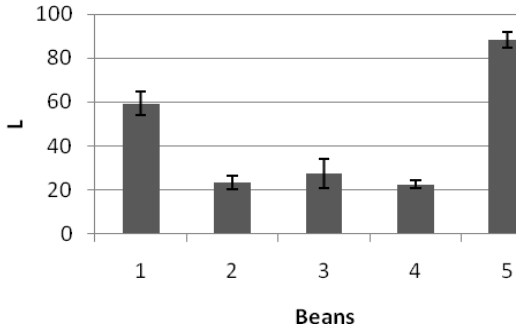


Fig. 7 L* values for investigated beans. 1-beige beans; 2-brown beans; 3-black/violet beans; 4-black beans; 5-white beans

Despite fact that all beans were kept in same conditions, significant differences ($F>F_{crit}$) were absorbed for crude protein content in beans, as shown in Fig. 8.

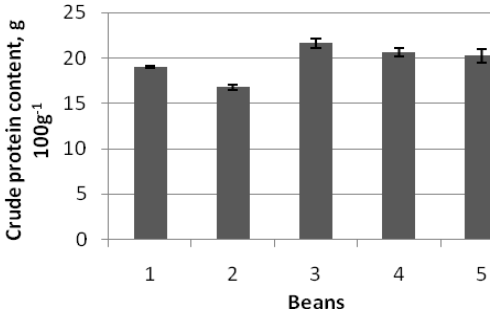


Fig. 8 Crude protein content of investigated beans g $100g^{-1}$. 1-beige beans; 2-brown beans; 3-black/violet beans; 4-black beans; 5-white beans

Less proteins is in brown beans (sample 2) – 16.80±0.30g $100g^{-1}$, but highest protein content were in black/violet beans (sample 3) – 21.60±0.50 g $100g^{-1}$. Beige (sample 1) beans had 19.00±0.10g $100g^{-1}$ crude protein on dry matter. Experimentally we have observed that beige (sample 1) and brown beans (sample 2) had significant differences from other beans, as black beans had 20.60±0.50g $100g^{-1}$ crude protein content, but white ones had 20.20±0.30g $100g^{-1}$ crude protein content, which were the only ones without significant differences ($p\leq0.05$).

Ash content in beans is presented in Fig. 9. Ash content in the same time less was for white beans (sample 5), only 4.00±0.02 g $100g^{-1}$, but highest were beige beans (sample 1) with 5.02±0.01 g $100g^{-1}$.

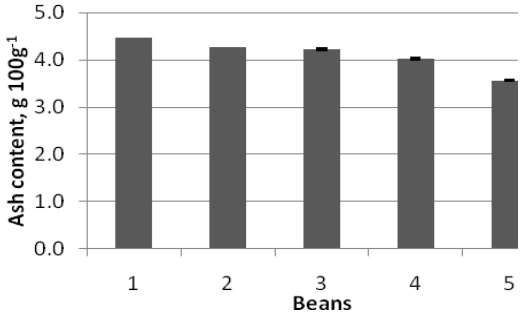


Fig. 9 Ash content in investigated beans g $100g^{-1}$. 1-beige beans; 2-brown beans; 3-black/violet beans; 4-black beans; 5-white beans

Compared all investigated samples we can summarized, that ash content in beans in sample 5 differed from ash content in another's investigated bean samples. Still data analysis shows significant differences ($F>F_{crit}$). As for brown (sample 2), black (sample 4) and black/violet beans (sample 3), ash content was 4.80±0.01g $100g^{-1}$, 4.52±0.04g $100g^{-1}$ and 4.74±0.02g $100g^{-1}$ accordingly. All data shows significant differences ($p\leq0.05$) except for brown (sample 2) and black/violet beans (sample 3), where differences are not significant.

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There can be seen differences in bean lightness, especially for white beans, where values are highest, but brown and black beans have no significant differences ($p<0.05$). Significant differences were found using statistical analysis with a and crude protein, but correlation was moderate (-0.55).

III. Conclusion

As a conclusion obtained results can be summarized that different coloured beans have various crude protein and ash content but it is not significantly different, except for brown and white beans.

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LieneStrauta,was born in Jelgava in 1987. She received her M.S. degree in Chemistry Latvia University at 2011. She is presently first course student of Ph.D. program Food Science of Latvia University of Agriculture. Thesis of PhD studies is "Suitability of legumes for dried breakfast". Scientific direction is to make new legume products and find a suitable packaging type for them.

Sandra Muizniece–Brasava, Dr. sc. ing., assistant of professor was born in Latvia, Gulbene at 1977. She has defended her Dr. Degree in Food Science in Latvia University of Agriculture at 2006. Thesis of PhD degree was "Poly-b-hydroxybuturate composite materials as environmentally friendly food packaging".Scientific direction is estimation of the novel and environment friendly food product packaging materials and packaging technologies. She has 40 published scientific publications (8 in SCOPUS database), 3 LR patents and 2 published books.

Ina Alsina, Dr.biol., assoc. professor of Faculty of Agriculture, Latvia University of Agriculture. was born in Latvia, Auce at 1960. Scientific interests are symbiotic nitrogen fixation in legumes, mainly in peas and beans. She has published more than 65 scientific publications(6 of them are indexed at SCOPUS database), author of 4 patents of Latvia.