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The change of protein and fat content in the beans seed covers during germination process

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Summary. The paper presents the results of studies on the percentage variation in protein and fat content in bean seed covers *Phaseolus vulgaris L.* of *Piękny Jaś* variety, during the germination process at a temperature of 20 °C ± 1 °C. The first samples were collected for analysis the next day from the start of the experiment while the others were taken every 24 hours during 5 consecutive days. The experiment showed an increase in fat content in seed covers (0.94 $g \cdot 100g^{-1}$ dm –1.35 $g \cdot 100g^{-1}$ dm) and simultaneous reduction in the protein content from 4.5 $g \cdot 100g^{-1}$ dm to 3.8 $g \cdot 100g^{-1}$ dm along with the increase in germination intensity. The analytical tests were terminated after 144 hours when the majority of seeds had germinated.

Key words: bean seeds, germination, crude protein, fat.

INTRODUCTION

Leguminous plants are considered as a source of protein with very high nutritional value. Peas, beans, broad beans and lentils contain more than 20% of proteins, while soybeans even 35-40%. Apart from the protein, legumes are the source of carbohydrates and fat rich in unsaturated fatty acids. Their presence makes beans recommended in the low cholesterol diet. Pulses are also rich in vitamins from the B group, particularly thiamine and niacin. In the fresh form, legumes are also considered as a good source of vitamin C and carotene. Both, dry and fresh pulses, provide significant amounts of such minerals as iron, copper, magnesium, calcium, potassium and sulfur [20]. Bean seed flour is also used as an additive to wheat flour in the production of instant noodles [24].

From a chemical point of view, fats are the natural organic compounds with varied structure, built from a carbon, oxygen and hydrogen. Fat content in leguminous seeds depends on their variety while it presence ensures the highest nutritional value [26]. Plant after drying (dry mass – dm) contains up to 98% of organic compounds. From the thousands of different substances, some are present in significant quantities in all tissues and cells, but most could be detected only by sensitive analytical methods. The variability of the individual substances is due to the metabolic processes testifying a given tissue vitality [5, 22].

Recent years require that plants resistant to drought should be grown, due to the fact that the effects of climate change are beginning to be felt around the world and drought starts to become a rising problem in a number of countries. Study on proteins commonly found in living organisms that belong to the large family of membrane integral proteins (MIP) and take part in the collection of water, demonstrated that three of them appeared in the germinating seeds after 60 hours since the process starts, i.e. after the main phase of water intake called the physical stage. What is more, a very high level of protein in the cell membrane, which could lead to gain an actual knowledge of the mechanism regulating water intake in the seeds growth and germination processes, was observed [6, 21].

Bean (*Phaselous vulgaris L.)* belongs to the species that in Poland plays an important role for nutritional and commercial reasons in crop production [13, 16]. Beans are consumed in various forms (canned, cooked, dried, frozen) being an important source of vitamins and minerals in the human diet.

The seeds quality and the possibility of a detailed assessment of the germination process are of great importance for agriculture, and for those industries that are closely related to it. The seeds are used for both, ground and greenhouse, production and require considerable financial and labour resources. Under these conditions, seed germination control is essential due to the fact that too low germination rate may lead to significant financial losses [10]. Studies have shown that germinating seeds are very rich in vitamins, thanks to the active enzymes that additionally resolve proteins, carbohydrates and fats on the simple compounds easily assimilated by the body.

Therefore, sprouts are not only healthy, but also easily digestible. It was also found that the sprouts are large reserves of minerals, mineral salts and other nutrients which make them far less caloric than the same seed [19, 23] and what is more are also an element of nutritional products for animals [25].

It is believed that the germination process, especially the first phase of water intake and seeds swelling as well as the biochemical processes during the seeds respiration are the source of energy that appears just after swelling process starts. Respiratory substrates are substances accumulated in them, among other things, fats and proteins [3]. Seed cover is the tissue covering the seed and protecting it that is why its structure and chemical composition play an important physiological role in germination process due to the different water and gas permeability [2, 3].

The aim of this study was to examine changes in the crude protein and fat content in bean seed covers, formed from the seedbud cover which genetic material is only of the stem origin, during *Phaseolus vulgaris L.* of *Piękny Jaś* variety germination.

GERMINATION PROCESS

Biological issues of seeds growth, development and germination in define environmental conditions are closely linked and controlled at the molecular, subcellular and cellular level as well as on the level of whole seed. Gradual reduction in the intensity of physiological processes during seed maturation could be observed while throughout the rest period latent life is maintained. Seeds are also able to resume rapidly the physiological processes during swelling and germination. Preserving vitality viable seeds might remain at latent stage for a long period of time. The standstill period (anabiosis) depends both on the genetic properties and environmental conditions. Dry seeds at latent stage are usually resistant to external factors, despite the biological preparation for further development, which is possible at any time, if only the access to water, proper temperature, oxygen and, for some species to light, are provided [11].

Germination is therefore perceived as a complex process built with partial phases that result in anatomical and morphological changes, observable without the use of a microscope [2]. It is assumed that germination process contains: water uptake, the cells elongation resumption, increase in the number of enzymes and reserve substances decomposition and mixing, increase in respiratory rate and in the intensity of cell division for different tissues [12].

The presence of proteins that are involved in regulating, among other things seed covers elasticity was detected in the seeds cell walls. Their modification is possible by deposition on their surfaces various types of organic compounds that include fats. Polymerized fatty acids coat the cell surfaces, leading to changes in the water conductivity during germination [3, 4].

MATERIAL PREPARATION FOR THE EXAMINATION, ACQUISITION OF THE EXPERIMENTAL DATA

For the tests bean seeds (*Phaselous vulgaris L.*) of *Piękny Jaś* cultivars were selected. In the experiment 1000 seeds were subjected to observation. Seeds were sown in trays on several layers of filter paper to ensure proper germination conditions by continuous supply of deionized water [17]. Before the experiment seeds, along with the tissue paper, were sterilized for 30 min at 70 °C and then cooled to the room temperature. Bean seeds germinated in a dark thermostatic chamber maintaining the temperature at a level of 20 °C ± 1 °C. On the basis of 300 seeds fundamental parameters characterizing their vitality were calculated: germination capacity Z_k and one seed germination time, so called Pieper time t_p. Remained seeds were used for analytical tests on protein and fat content. The use of deionized water prevented providing the seeds with macro- and microelements that could affect or change the content of the analyzed variables [7].

It is believed that the germination process, especially the first phase of water uptake and seed swelling as well as the biochemical processes during the respiration are the source of energy that appears early after the seeds swelling initiation. Respiratory substrates are accumulated backup substances, among other things, fats and proteins found in various parts of the seed, including the shell [3].

The germination capacity of seeds is usually determined by physiological methods which are used to observe the germination process under laboratory conditions. Germination capacity Z_k can be expressed as the percentage of seeds which germinate normally under given conditions over a sufficient period of time:

$$Z_k = \frac{n}{n_c} \cdot 100\%, \tag{1}$$

where: n is the number of sprouted seeds, n_c is the total number of seeds. Germination rate E_k, i.e. the percentage of seeds which germinated normally under the set conditions and in a given period of time, usually the moment of the first recalculation of germinating seeds, is described by the following equation:

$$E_k = \frac{n(t_1)}{n_c} \cdot 100\%, \tag{2}$$

where: $n(t_1)$ is the number of sprouted seeds during the first recalculation; n_c is the total number of sown seeds. Seed viability may also be expressed by Pieper's coefficient t_p [11], i.e. the average time required for one seed to germinate:

$$t_p = \frac{\sum_{i=1}^{n} n_i \cdot t_i}{\sum_{i=1}^{n} n_i}, \tag{3}$$

where: n_i is the number of seeds germinating within a given time interval: i=1,2,3,…,n; t_i is the seed germination time.

Figure 1 shows the experimental and calculated curve drawn for the bean seeds germination process, along with the marked time of seed cover collection for the analysis. Pieper time t_p [11, 15, 18] calculated on the basis of the obtained germs accounted for 212.8 hours. Germination curve is characterized by typical shape with a well-defined saturation after about 340 hours. The calculated germination capacity Z_k reached 87.8%.

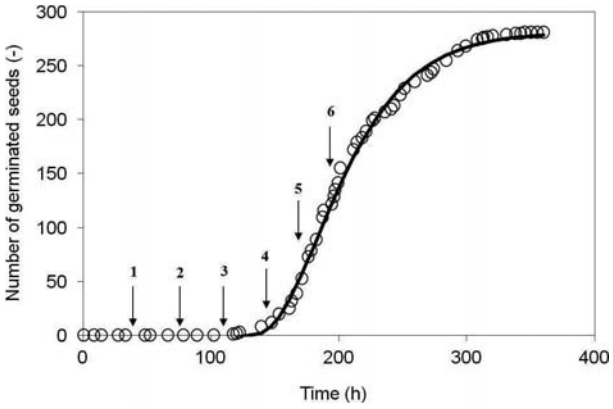

Fig. 1. Experimental (○) and calculated (solid line) germination curves for bean seeds at the temperature of 20°C.

Black arrows indicate the time when seed covers were collected for the analysis:

$$n(t) = n_k \cdot \left(1 - \frac{\alpha \cdot e^{-\lambda_1 (t-t_0)} + \beta \cdot e^{-\lambda_2 (t-t_0)} + \gamma \cdot e^{-\lambda_3 (t-t_0)}}{\alpha + \beta + \gamma}\right). \quad (4)$$

The germination process can be described mathematically with the use of a simulation model [8,9] based on the assumption that germination has three distinctive stages: physical, biochemical and physiological. It has been assumed that the germination process involves gradual evolution through the successive stages at a given level of probability. In its analytical form, the model can be expressed [9] by the equation (4), where:

$$\alpha = \lambda_2 \cdot \lambda_3 \cdot (\lambda_2 - \lambda_3), \quad (5)$$

$$\beta = \lambda_3 \cdot \lambda_1 \cdot (\lambda_3 - \lambda_1), \quad (6)$$

$$\gamma = \lambda_1 \cdot \lambda_2 \cdot (\lambda_1 - \lambda_2). \quad (7)$$

Parameters λ_1, λ_2, λ_3 indicate the probability of the seed's transition from one stage to another; *n(t)* is the number of seeds sprouted at a given time; n_k is the final number of sprouted seeds; t – is the germination time; t_0 – is the time required for the seed to emerge from the latent development stage and to enter the sprout formation stage. For the analyzed germination curve the best fit has been obtained with the following parameters: $\lambda_1 = \lambda_2 = \lambda_3 = 10^{-4}$ s^{-1}, t_0=130 h, n_k =281. The fact that probability parameters are equal indicates that each stage of the seeds evolution is also equally present in the germination process.

CRUDE PROTEIN AND FAT CONTENT DETERMINATION

Protein content was determined with the Kjeldahl method application in the Kjel-Foss Automatic (A / S Foss Electric, Denmark) device according to AOAC 976.05 while the fat content determined by extraction- gravimetric method with the Soxtec HT-6 use in the Central Analytical Laboratory of the University of Life Sciences in Lublin.

The analytical investigation was carried out in parallel with studies on germination in the subsequent days of the experiment which were marked with black arrows in Figure 1. Examined seeds were randomly selected and its cover was removed and next dried for 3 h in thermostatic oven until dry mass was obtained. Then dried material was placed in a mortar, covered with liquid nitrogen at about -186 °C and grinded to a fine dust. Then three dust samples of 0,5g were weighed with an accuracy of ± 0.01 g on the analytical balance WAS 160/C/2. Prepared samples were then poured into a specially-marked 1.5 ml eppendorfs. Each sample was subjected to3 measurements.

Table 1. Fat (a) and protein (b) content in bean seeds during germination in 20°C, marked in three tests

a)

Time (hours)	Fat content (g·100 g^{-1} dm)			
	I test	II test	III test	Average
24	0.89	0.82	0.85	0.85
48	1.05	0.87	0.88	0.93
72	0.80	0.89	0.89	0.86
96	0.89	0.84	0.87	0.87
120	1.12	1.01	1.06	1.06
144	1.89	1.82	1.88	1.86

b)

Time (hours)	Protein content (g·100 g^{-1} dm)			
	I test	II test	III test	Average
24	7.10	7.40	7.44	7.31
48	6.94	6.83	7.00	6.93
72	6.64	6.81	7.20	6.88
96	6.45	6.16	6.35	6.32
120	5.86	5.67	5.58	5.70
144	5.64	5.78	5.69	5.70

RESULTS AND DISSCUSSION

The protein content in whole seeds dry mass for dwarf varieties of common bean: Toffi, Tip Top, Nigeria and Augusta marked by many researchers ranged from 23.96 to 29.85 g·100g^{-1} dm. The fat content in seeds of these varieties was lower and changed from 1.54 to 1.78 g·100g^{-1} dm [1, 4, 10, 14].

Whereas protein content in seed covers is usually much lower. Bean seeds are characterized by protein content values in the range of 5 g·100g^{-1} dm [23], which was confirmed in the presented work.

Table 1 shows numerical values of measured variables for consecutive days of experiment as well as their average value. In table 1 mean difference between the maximum and minimum values at three tests for fat (a) and protein (b) in germinated bean seed covers subjected to germination at the 20 ° C are presented. The fat content in beans covers during germination in deionized water was twice lower than in the whole seeds (0.85 to 1.86 g·100g^{-1} dm), while protein content was considerably lower (7.31- 5.70 g·100g^{-1} dm).

Figure 2 shows the fat content determined by extraction- gravimetric method for dry bean seed covers naturally moistened during the germination process and the maximum deviation from the average values. First marking for seed samples was performed after 24 hours of the beans were sowed in trays on the constantly humidified filter paper. Average fat content determined in three repetitions reached 0.85 g·100g^{-1} dm. Successive days of the research indicated a systematic increase in the fat content. The highest increase occurred between 5th and 6th germination day, when the fat content raised from 1.06 g·100g^{-1} dm to 1.86 g·100g^{-1} dm.

Average protein content value in samples taken from seeds at the same time intervals determined by the Kjeldahl method decreased steadily from 7.31 g·100g^{-1} dm on the first day to 5.7 g·100g^{-1} dm on the last day of analysis (see Fig.3). Recent determination of the protein in experiment 144th hour indicated the same value as in the previous day (120 hrs.).

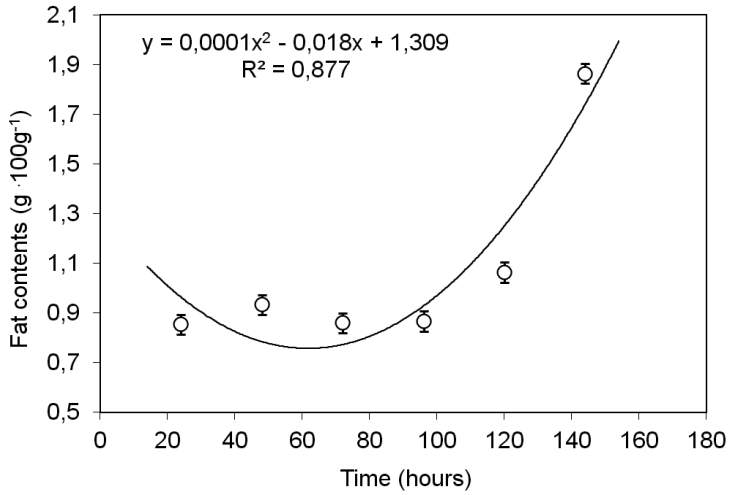

Fig. 2. Fat contents in bean seed cover during germination process. Error bars indicate the average difference between results and the average calculated for three samples

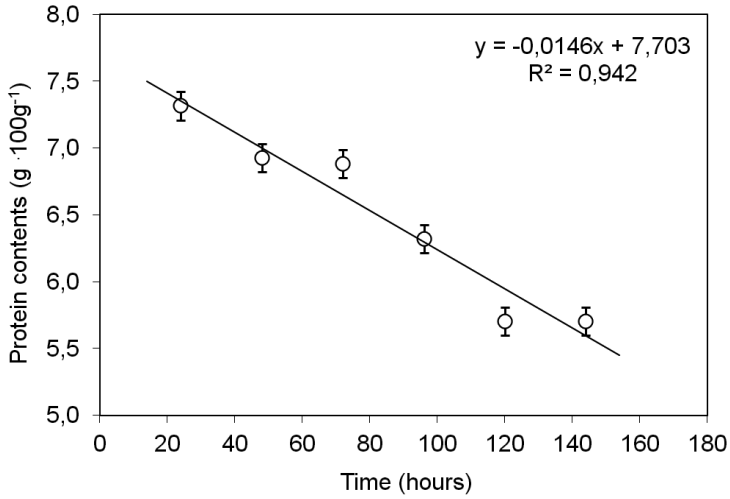

Fig. 3. The protein content in bean seed cover during germination process. Error bars indicate the average difference between results and the average calculated for three samples

In the 72th germination hour, when almost all seeds had completed the swelling stage, protein content in the cover decreased steadily from 6.88 g·100g^{-1} dm (72th hour) to 6.32 g·100g^{-1} dm in the 120th hour An identical value was obtained for seed cover samples on the last day of measurement (144th germination hour). Observed decrease in the protein content was consistent with the highest intensity of germination (Pieper time t_p= 140.8 hours).

Although the germination process took more than 300 hours, chemical analyzes were completed in 144 hours, as most seeds sprout achieving the final effect of the germination process.

CONCLUSIONS

1. The fat content determined with the use of extraction-gravimetric method in seed cover remained steady during first 96 hours at the level of 0.9 g·100g^{-1} dm -1.14 g·100g^{-1} dm. In the next two days (48 hours) fat content increase up to 1.86 g·100g^{-1} dm was observed. The fat content in seeds with visible sprout was doubled in relation to the value determined on the first day of germination.
2. Crude protein content, set with the use of the Kjeldahl method, decreased from 7.31 g·100g^{-1} dm in 24th hour of germination to 5.70 g·100g^{-1} dm – the value defined in 120th experiment hour. This value was maintained for another day which was proved by the protein content measurement in 144th germination hour.
3. Starting from 72th hour of germination a significant increase in fat content could be observed while the protein content strongly decreased (6.88 g·100g^{-1} dm - 5.70 g·100g^{-1} dm). It may indicate that the backup substances causing the seed embryo's growth, cover breakdown and seedling emergence were depleted.

REFERENCES

1. **Berrios J.J., Swanson B.G., Cheong W.A. 1999:** Physico-chemical characterization of stored black beans (Phaseolus vulgaris L.). Food Res. Int., 32, p. 669-676.
2. **Berlyn G.P. 1972:** Seed germination and morphogenesis. In: Kozłowski, Seed biology, Acad. Press, N.Y. London, vol. 1, p. 223-312.
3. **Bewley J.D., Black M. 1972:** Physiology and biochemistry of seed in relation togermination. Vol. 1,2. Springer Verlag, Berlin-Heidelberg-New York.
4. **Candela M., Astiasaran I., Bello J. 1997:** Cooking and warm-holding: effect on general composition and amino acids of kidney beans (Phaseolus vulgaris), chickpeas (Cicer arietinum), and lentils (Lens culinaris). J. Agric. Food Chem., 45, p. 4763-4767.
5. **Cone W.J. 1982:** The Escape From Photocontrol of Seed Germination of Arabidopsis Thaliana. Arabid. Inf. Serv.(19) p. 35.
6. **Gattolin S., Sorieul M., Frigerio L. 2011:** Mapping of tonoplast intrinsic proteins in maturing and germinating arabidopsis seeds reveals dual localization of embryonic tips to the tonoplast and plasma membrane. Molecular Plant 4, p. 180-189.
7. **Gładyszewska B. 2007:** Method of testing of selected mechanical properties of thin biological materials (in Polish), habilitation thesis AU in Lublin, p. 325.
8. **Gładyszewska B. 1998:** Ocena wpływu przedsiewnej laserowej biostymulacji nasion pomidorów na proces ich kiełkowania. Rozprawa doktorska. Lublin.
9. **Gładyszewska B., Koper R.:** Symulacyjny model procesu kiełkowania nasion w ujęciu analitycznym. Inżynieria Rolnicza, 7, p. 59-63
10. **Granito M., Frias J., Doblado R., Guerra A., Champ M. 1960:** Nutritional improvement of beans Phaseolus vulgaris) by natural fermentation. Eur. Food Res. Technol., 2002, 214, p. 226-23.
11. **Grzesiuk S. Kulka S. 1981:** Fizjologia i biochemia nasion. PWRiL., Warszawa.
12. **Kramer P.J., Kozłowski T.T. 1980:** Physiology of trees. MC Graf-Hill N.Y. Toronto, London.
13. **Jasińska Z., Kotecki A. 1993:** Rośliny strączkowe. PWN. Warszawa.
14. **Martín-Cabrejas M.A., Sanfiz B., Vidal A., Mollá E., Esteban R., López-Andréu F.J. 2004:** Effect of fermentation and autoclaving on dietary fiber fractions and antinutritional factors of beans (Phaseolus vulgaris L.). J. Agric. Food Chem., 52, p. 261-266.
15. **Noogle G.R., Fritz G.L. 1976:** Introductory plant physiology. Prentice-Hall, New Jersey, p. 264-265.
16. **Podleśny J. 2005:** Rośliny strączkowe w Polsce- perspektywy uprawy i wykorzystanie nasion. Acta Agrophys. 6 (1), p. 213-224.
17. Polska Norma PN-96/R-65950. Materiał siewny. Metody badania nasion.
18. **Pieper G., Eggebrecht H. 1995:** Das Saatgut. Wydanie II, Berlin.
19. **Shimelis E.A., Rakshit S.K. 2005:** Proximate composition and physico-chemical properties of improved dry bean (Phaseolus vulgaris L.) varieties grown in Ethiopia. Libens Wiss Technol. 38, p. 331-338.
20. **Szczygieł A., Bułhak–Jachymczak B. 1975:** Podstawy fizjologii żywienia. PZWL, Warszawa.
21. **Taormina P.J., Beuchat L.R., Slutsker L. 1999:** Infections Associated with Eating Seed Sprouts: An International Concern. Emerging Infectious Diseases 5, p. 626.
22. **Vargas-Torres A., Osorio-Díaz P., Tovar J., Paredes-López O., Ruales J., Bello-Pérez L.A. 2004:** Chemical composition, starch bioavailability and indigestible fraction of common beans (Phaseolusvulgaris L.). Starch/ Stärke, 56, p. 74-78.
23. **Wu X. 2002:** Correlation of physic-chemical characteristics in the seed covers and canning quality in different dark red kidney bean (Phaseolus Vulgaris L.) cultivars. MSC thesis University of Wisconsin-Stout, Research paper.
24. **Wojtowicz A. 2008:** Influence of legumes addition on proceeding of extrusion-cooking process of precooked pasta. Teka Kom. Mot. Energ. Roln. – OL.PAN, 8a, p. 209-216.
25. **Young A.W., Grummer R.H., Hirschinger C.W. 1977:** Using cullkidney beans in swine protein supplement. Research Report No. 2892, University of Wisconsin-Madison, College of Agricultural and Life Sciences.

26. **Ziemlański Ś., Budzyńska-Topolowska J. 1991:** Tłuszcze pożywienia i lipidy ustrojowe. Wydawnictwo Naukowe PWN. Warszawa.

ZMIANA ZAWARTOŚCI BIAŁKA I TŁUSZCZU
W OKRYWIE NASIENNEJ FASOLI
PODCZAS KIEŁKOWANIA

S t r e s z c z e n i e . W pracy przedstawiono wyniki badań procentowej zmienności zawartości białka ogółem i tłuszczu w okrywach nasion fasoli Phaseolus vulgaris L. odmiany Piękny Jaś, w trakcie procesu kiełkowania przebiegającego w temperaturze 20^{o}C ±1^{o}C. Pierwsze próbki do analizy pobrano następnego dnia od chwili rozpoczęcia eksperymentu, następne pobierano co 24 godziny przez 5 kolejnych dni. Badania wykazały zwiększenie zawartości tłuszczu (0,89 g·100g^{-1} s.m. – 1,86 g·100g^{-1} s.m.) oraz zmniejszenie zawartości białka w okrywach z 7,31 g·100g^{-1} s.m. do 5,70 g·100g^{-1} s.m. wraz ze wzrostem intensywności kiełkowania. Badania analityczne zakończono po 144 godzinach, gdy większość nasion zakończyła już kiełkowanie.

S ł o w a k l u c z o w e : nasiona fasoli, kiełkowanie, białko surowe, tłuszcz.