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INFLUENCE OF SAMPLE WEIGHT ON AMOUNT OF EXTRACTED PROTEIN FROM PELLETED SAMPLES

UTICAJ MASE UZORKA NA KOLIČINU EKSTRAHOVANIH PROTEINA IZ PELETIRANOG MATERIJALA

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ABSTRACT

Protein is one of the major components of the pe1leted animal feed. Thus, it is very important to properly access protein quality and quantity. In this paper ground corn is pelleted on a flat die pellet press. The extraction buffer (0.125 M Tris-HCl at pH 6.8 containing 10% β-mercaptoethanol, 4% sodium dodecyl sulfate, 20% glycerol) was used for extraction of protein from pelleted samples. Additionally, Lab-on-a-chip (LoaC) electrophoresis is used for qualitative and quantitative characterisation of protein from pelleted samples. Sample weights used for analysis were 30, 40 and 50 mg. Relative protein concentration (mass of protein in the sample per sample weight) was highest for sample weight of 30 mg, and it was 20,598.42 ppm, while concentrations of sample weights of 40 and 50 mg were 5,976.46 and 5,226.94 ppm, respectively.

Key words: *pelleting, protein analysis, LoaC electrophoresis.*

REZIME

Proteinska komponenta je jedna od najzastupljenijih komponenti u peletiranoj hrani za životinje. Ona ima značajnu ulogu u peletiranoj hrani kako sa nutritivnog aspekta, tako i sa aspekta njenog pozitivnog uticaja na fizički kvalitet peleta. Stoga je veoma bitno da se pravilno odredi kvalitet i količina proteina u uzorku. U ovom radu kukuruz samleven na mlinu čekićaru sa sitima otvora 2 mm i kondicioniran u parnom kondicioneru na temperaturi od 80°C, a zatim peletiran na pelet presi sa ravnom matricom. Nakon toga peletirani kukuruz je samleven i iz njega su ekstrahovani proteini. Za ekstrakciju proteina iz peletiranih uzoraka korišćen je ekstrakcioni pufer sledećeg sastava: 0.125 M Tris-HCl pri pH 6,8, 10% β-merkaptoetanola, 4% natrijum dodecil sulfata, 20% glicerola. Lab-on-a-chip (LoaC) elektroforeza je korišćena za kvalitativnu i kvantitativnu karakterizaciju proteina iz peletiranih uzoraka. Separacija na bazi čipova izvođena je na Agilent 2100 bioanalajzeru. Mase uzoraka upotrebljenih za analizu proteina bile su 30, 40 i 50 mg. Nakon ekstrakcije određena je relativna koncentracija proteina (masa proteina u uzorku po masi uzorka) Relativna koncentracija proteina je bila najveća za masu uzorka od 30 mg i iznosila je 20598,42 ppm, dok je relativna koncentracija proteina za mase uzoraka od 40 i 50 mg bila 5976,46, odnosno 5226,94 ppm.

Ključne reči: *peletiranje, analiza proteina, LoaC elektroforeza.*

INTRODUCTION

Most of the animal feed fed to broilers is in the pelleted form. Pelleting process denotes process in which bulk or powder material is forced through a die with specific dimensions, and cut at the outlet of the die. Results of this process are pellets, usually cylindrically shaped, with the diameter nearly equal to the diameter of die openings. Despite high investment and production costs, pelleting is widely used process. Reason for this is improving handling characteristics of pelleted product and improving nutritional and microbial quality of animal feed mixture. Pelleting improves handling characteristics in terms of increasing bulk density and flowability of the product, and stability of the mixture by reducing segregation of the components (*Stevens, 1987; Pfost 1963, Čolović et al., 2010)*. Obtained pellets should have quality which is enough to stand rigors of transport and handling which require pellets of certain integrity without fines produced by attrition stresses. On the other hand, production costs should be the least possible in terms of energy consumption and wear. Most of pellet presses used in animal feed industry have ring die design, in which the die rotates around the fixed rollers. Beside ring die pellet press, flat-die press is also used. In this press horizontal die is static and vertical rollers are rotated. *(Thomas et al., 1997; Pfost, 1971, Sredanović et al., 2005)*

Complete process of production pellets involves milling of feed stuffs, mixing of all components, steam conditioning, pelleting and cooling of pellets. Hammer and roller mills are usually used for milling of feed stuffs. By choosing screens of different size openings particle size distribution of the material could be adjust. Particle size distribution could have influence on quality of final product, likewise on specific energy consumption in the process (*Thomas and van der Poel, 1996)*. Steam conditioning process, often called as a pre-processing of the material to be pelleted, is a process of converting mixed mash with use of heat, water, pressure and time, to a physical state which is more suitable for compaction of feed mash. Generally, the purpose of steam conditioning is to prepare product for pressing. Steam conditioning increases production capacity and, in the same time, affects physical, nutritional, and hygienic quality of produced feed. Steam conditioner is a continuous or batch mixer in which adequate absorption of steam is provided *(Sredanović and Lević, 2000, Vukmirović et al., 2010*).

Application of heat and water in process of production pellets process leads to changes of components. Heat and water inducing wide range of physical and chemical changes, such as thermal softening of feed, denaturation of proteins, gelatinization of starch, etc. Chemical and physical changes influence nutritional quality, in terms of better feed intake and improved nutritional value *(Maier and Gradecki, 1992, Winowiski, 1985, Skoch et al., 1983)*.

For accessing quality of components various methods are used. Starch gelatinization could be determined by enzymatic methods, rheological methods, differential scanning calorimetry, etc. (*Lankhorst et al., 2006, Yoshimura et al., 1999*). Protein

quality is often described by solubility methods, such as Protein Dispersibility Index, Nitrogen Solubility Index, Protein Solubility in KOH, etc. *(Palić et al., 2009)*. LoaC electrophoresis of protein extracts is increasingly used in separation and quantification of protein component in various types of samples (*Torbica et al., 2010, Tomić and Torbica, 2011*).

In this paper buffer that was previously used for the extraction of wheat proteins (*Kasarda et al., 1986*) was used for protein extraction from pelleted corn. Considering that the analyzed matrix differs from wheat, in order to improve the protein extraction conditions, different sample weight of the analyzed sample was tested.

MATERIAL AND METHOD

Maize sort "ZP 434" grown in northern Serbia was used in this experiment. Maize kernels were milled in hammer mill (ABC Engineering, Pančevo, Serbia) at capacity of 50 kg/h. Sieve with openings' size of 2 mm was inserted in hammer mill. After milling, maize was transported to batch steam conditioner (Muyang, China). Steam was dosed to material in steam conditioner until temperature of 80°C was reached. Conditioned material was pelleted at laboratory pellet press (Figure 1) with flat die with sieve openings' size of 6 mm (ratio between length and diameter 1:4).

Fig. 1. Laboratory pellet press

Cold pellets were grounded. Mass of samples was set to be 30, 40 and 50 mg. For extraction of proteins extraction buffer was prepared. Ingredients of extraction buffer are listed in Table 1.

Table 1. Ingredients of extraction buffer

Ingredient	Quantity
0.125 M Tris-HCl (pH = 8)	66%
β-mercaptoethanol	10%
sodium dodecyl sulfate	4%
glycerol	20%

Extraction buffer was mixed with H_2O (175 µL : 175 µL). Samples were mixed with extraction buffer/H_2O mixture, heated for 5 min at temperature of 100°C, then left for 20 min at room temperature and vortexed. Supernatant, in which total soluble proteins were present, was used in LoaC electrophoretic analysis.

Agilent 2100 bioanalyzer (Agilent Technologies, USA) with Protein 230 Plus LabChip kit was used for chip-based protein separations (Figure 2). Protein 230 software assay was used for displaying results. All samples were analyzed in triplicate.

Fig. 2. Agilent 2100 bioanalyzer

STATISTICA 8.0, statistical analysis system (Statsoft, USA) was used for analysis of variance – ANOVA. The level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Concentration of proteins in extracts from different mass of pelleted samples is shown in Fig. 3. It can be seen that highest concentration of extracted proteins was obtained for sample weight of 30 mg (20598.42 ppm). Significantly ($p > 0.05$) lower concentration of extracted proteins was achieved for sample weight of 40 mg (5976.46 ppm). Concentration of extracted proteins from sample weight of 50 mg was 5226.94 ppm, which was significantly ($p > 0.05$) lower when comparing with extract from sample weight of 30 mg, and slightly lower, when comparing with extract from sample weight of 40 mg.

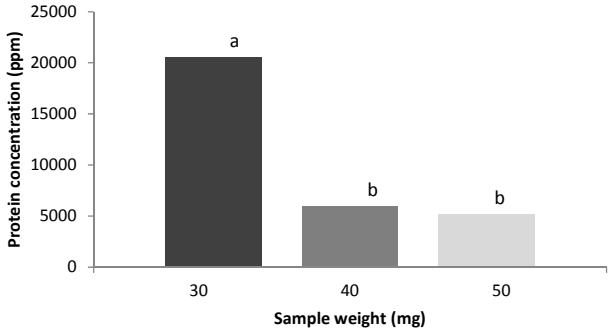

Fig. 3. Concentration of proteins in extracts
[a]Columns with different letters are significantly different at the 5% level

Protein concentration in extracts of different sample weights in dependence of molecular weight of protein is shown in Fig. 4. Concentration of single protein fractions was highest in extract from sample weight of 30 mg, which also can be seen on the electropherogram shown in Fig 5. The number of separated protein fractions was slightly lower for sample weights of 40 and 50 mg. However, concentration of individual fractions was significantly ($p > 0.05$) lower when comparing with sample weight of 30 mg. These differences in electrophoretic profiles of samples may be related to the fact that the increased sample weight of the sample resulted in incomplete extraction of corn proteins.

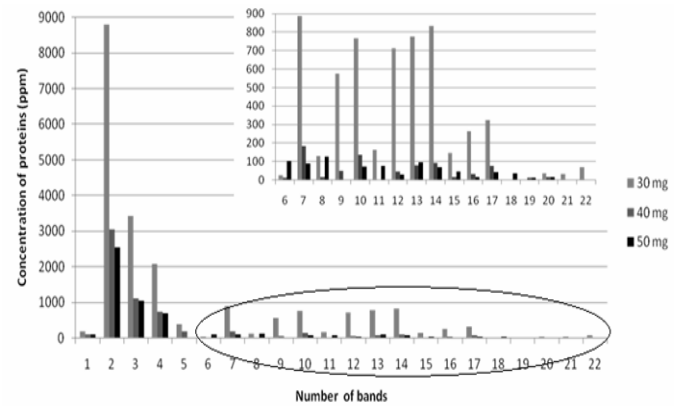

Fig. 4. Protein concentration according to molecular weight

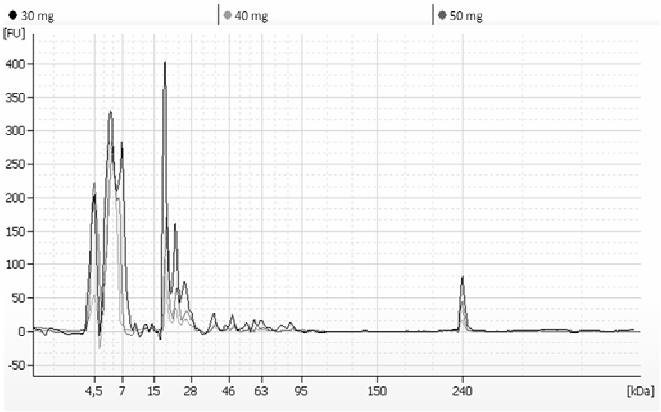

Fig. 5. LoaC profiles of extracted proteins from pelleted samples

CONCLUSION

Results in this paper have shown that electrophoresis could be considered as a very effective technique for the analysis of proteins in pelleted corn samples. Nevertheless, weight of samples highly influenced qualitative and quantitative differences between analyzed samples. Higher sample weights caused incomplete extraction of proteins from samples, which in turn induced differences in yield of protein extract and differences in electrophoretic profiles of analyzed samples.

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REFERENCES

Čolović, R., Vukmirović, Đ., Matulaitis, R. Bliznikas, S., Uchockis, V. Juškiene, V., Lević, J. (2010). Effect of die channel press way length on physical quality of pelleted cattle feed, Food and Feed Research 37 (1), 1-6.

Kasarda, D., Furlath, K., Thrasher, K. (1986). Sodium dodecyl sulfate polyacrylamide gel electrophoresis of wheat grain proteins from selected wheat varieties and FGIS wheat class samples: A survey of samples from the hardness series. Manuscript, Western Regional Research Center, Agricultural Research Service, USDA, USA.

Lankhorst, C., Tran, Q.D., Havenaar, R., Hendriks, W.H., van der Poel, A.F.B. (2006). The effect of extrusion on the nutritional value of canine diets as assessed by in vitro indicators. Animal Feed Science and Technology, doi:10.1016/j.anifeedsci.2006.11.015.

Maier, D.A., Gradecki, J. (1992). Feed mash conditioning field case studies. American Society of Agricultural Engineers, International winter meeting, Nashville, USA, paper no. 92-1541.

Palić, D., Coetzee, E. (2009). Postupak za korišćenje rastvorljivosti proteina kao indikatora termičkog tretmana punomasne soje, Acta Periodica Technologica, 40, 71-77.

Pfost, H.B. (1971). Equipment and techniques in starch gelatinization. Feedstuffs, February 27, 24.

Pfost, H.B. (1963).Testing the durability of pelleted feed, Feedstuffs, March 23, 66-68.

Skoch, E.R., Binder, S.F, Deyoe, C.W., Alee, G.L., Behnke, K.C. (1983). Effects of pelleting conditions on performance of pigs fed a corn-soybean meal diet. J. Anim. Sci., 57(4), 922-928.

Sredanović S., Lević, J. (2000). Conditioning: An important step in feed production. Journal on processing and energy in agriculture / PTEP, 4 (3-4), 82 - 84.

Sredanovic S., Lević J., Đuragić O. (2005). Identification of feed raw material hazard properties. Journal on processing and energy in agriculture / PTEP, 9 (5), 120-123.

STATISTICA (Data Analysis Software System), v.8.0 (2006). Stat-Soft, Inc, USA (www. statsoft.com)

Stevens, C.A. (1987). Starch gelatinization and the influence of particle size, steam pressure and die speed on the pelleting process. Ph.D. Thesis Kansas State University, USA.

Thomas, M., van der Poel, A.F.B. (1996). Physical quality of pelleted animal feed 1. Criteria for pellet quality. Animal Feed Science Technology, 61, 89-112.

Thomas, M., van Zuilichem, D.J., van der Poel, A.F.B. (1997). Physical quality of pelleted animal feed. 2. contribution of processes and its conditions. Animal Feed Science Technology, 64, 173-192.

Tomić, J., Torbica, A. (2011). Evaluation of technological quality of wheat using Lab-on-a-Chip electrophoresis, Food and Feed Research, 38 (2), 63-69.

Torbica, A. Živančev, D. Nikolić, Z. Đorđević, V. Nikolovski, B. (2010). Advantages of the Lab-on-a-Chip Method in the Determination of the Kunitz Trypsin Inhibitor in Soybean Varieties. Journal of Agricultural and Food Chemistry, 58, 7980-7985.

Vukmirović, Đ., Ivanov, D., Čolović, R., Kokić, B., Lević, J., Đuragić, O., Sredanović, S. (2010). Effect of steam conditioning on physical properties of pellets and energy consumption in pelleting process. Journal on processing and energy in agriculture / PTEP, 14 (2), 106-108.

Winowski, T. (1985). Optimizing pelleting temperature. Feed Management, 36, 28-33.

Yoshimura, M., Takaya, T., Nishinari, K. (1999). Effects of xyloglucan on the gelatinization and retrogradation of corn starch as studied by rheology and differential scanning calorimetry, Food Hydrocolloids, 13 (2), 101-111.

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