BY-PRODUCTS OF BLACK (BRASSICA NIGRA) AND WHITE (SINAPIS ALBA) MUSTARD SEED PRODUCTION AS ANIMAL FEED - POSSIBILITIES AND HAZARDS

NUSPROIZVODI SEMENSKE DORADE CRNE (BRASSICA NIGRA) I BELE (SINAPIS ALBA) SLAČICE KAO HRANA ZA ŽIVOTINJE – MOGUĆNOSTI I OPASNOSTI

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ABSTRACT

The agri-food industry generates thousands of tons of by-products such as skins, peels, seeds, leaves and other inedible fractions. Such by-products usually cause environmental issues due to their large amounts and high water activity promoting microbial development. Food by-products can be readily recycled and utilized as a source of fuel, feed and fertilizer. Therefore, it seems logical and feasible to turn food by-products into animal feeds. The purpose of this study is to produce a new animal feed compound by extruding by-products of black and white mustard seeds production. After extrusion, the feed compound obtained was dried and stored in two manners: under ambient conditions and in a climate chamber under accelerated conditions of high temperature and air humidity. Our objective was to examine the oxidative stability of the feed compound produced, and to compare the properties of black and white mustard seed extrudates.

Key words: Brassica Nigra, Sinapsis Alba, by-products, extrusion, storage stability.

REZIME

Semenska proizvodnja stvara ogromne količine prehrambenog otpada svake godine, kao što su polomljenia zrna, ljuske, nejestivi delovi biljke, kao i delovi neadekvatnih senzornih i nutritivnih karakteristika. Jednostavan način za smanjenje količine semenskog “otpada” se određuje u vezi sa nosilacima tečnosti i parnovodnim čimbenicima. S obzirom na samo poreklo sirovine, kao najlogičniji i najefikasniji način za pristup odbačenim nusproizvodima imaju ekstrudirana nusproizvodi. S obzirom na poznate karakteristike, načine pristupljenja i vrednosti ovih komponenta, tečnosti i parnovodnosti, kao i načine u kojima se ekstrudirani nusproizvodi istražuju i proizvedeni ekstrudati pokazuju značajan potencijal kao novo hranivo za životinje. Visok sadržaj proteina, a u isto vreme i mali sadržaj masnih kiselina, čine ekstrudatede nusproizvode slabe u sebi bez nesigurnosti u ishrani životinja.

Ključne reči: Brassica Nigra, Sinapsis Alba, krušak, nusproizvodi, extrudiranje, skladišna stabilnost.

INTRODUCTION

According to the definition provided by the United States Environmental Protecting Agency (USEPA), food waste, raw or cooked, is any food substance discarded, intended, or required to be discarded (EPA, 2016). Food wastes are residues of high organic load in both liquid and solid forms, which usually remain after the processing of raw materials into foodstuffs. The fact that these substances are removed from the production process as undesirable materials defines them as “wastes” in most European legislations (Commission Regulations 442/1975/EEC, 1975). However, these “wastes” can potentially be reused inside the food chain and, for this reason, the term “food by-products” is increasingly used among the experts and scholars in order to emphasize that they can be raw materials for developing new products with an economic value (Gustavsson et al., 2013).

Nowadays, food “wastes” are referred to as a source of valuable nutritive components and have a great potential to be used for feeding the world’s fast growing population in the 21st century. The idea of using food by-products in such fashion originates from the extensive amounts of food related materials that are discarded worldwide, and the existing technologies
which promise the recovery, recycling and sustainability of high-added value ingredients inside the food chain (Galanakis, 2012). By-products represent about one-third of the poultry ration and about one-seventh of the ration for growing and fattening swine in the United States. They are also important in feeding beef and dairy cattle (Becker, 2008).

Mustard plants belong to the Brassicaceae family and possess seeds which are mainly used in food processing. Common types of mustard are white (yellow) mustard (Sinapis alba), brown mustard (Brassica juncea) and black mustard (Brassica nigra). Mustard plants have a rich chemical composition and their seed flour is widely used in food processing (Wanasundara, 2008; Abul-Fadl et al., 2011). Mustard is also used for its spicy flavour, produced from the hydrolysis of glucosinolates by myrosinase enzymes (Wanasundara, 2008). Mustard seed is widely used as a spice. However, its advantageous chemical composition and relatively low price offer wide possibilities for utilization as additives in human food and in animal feed (Wanasundara, 2008; Abul-Fadl et al., 2011).

In the mustard seed production, the seed is cleaned after harvesting in order to remove impurities, as well as broken and immature seeds which do not correspond to the required seed quality. The cleaning process separates the “waste” which is still rich in protein and fat (Kormanjaš et al., 2016). With regard to the considerations stated above, the purpose of this study is to determine the chemical composition of the new feed compound produced by extruding by-products of black and white mustard seed production, and to compare the properties of black and white mustard seed extrudates during storage.

MATERIALS AND METHOD

By-products of white (Sinapis alba) and black (Brassica nigra) mustard seed production were obtained from the Institute of Field and Vegetable Crops, Novi Sad, Serbia. By-products of black and white mustard seeds (BMS and WMS) production were extruded using a laboratory single screw extruder OEE 8 (AMANDUS KAHL GmbH & Co. KG, Germany). Before extrusion, the material was milled using a laboratory hammer mill (ABC Inženjering, Pančevo) featuring sieves with an opening diameter of 4 mm. The extrusion process was applied in order to increase the microbial safety of the products, as well as to enhance the digestibility of the material (Vukmirović et al., 2011). The following parameters were determined in the extrudates produced: crude protein, crude fat, moisture, ash and crude fibre content, as well as the fatty acid (FA) composition.

The moisture content of the samples was determined using the gravimetric AOAC Method 950.46, also known as the “oven dry” method, whereas the crude ash content was determined using the standard AOAC Method 942.05. The crude protein content was determined using the Kjeldahl method (according to the AOAC 978.04 Method), the crude fiber content was determined using the AOAC 978.10 Method (AOAC, 2000), and the total fat content using the Soxhlet procedure (as explained in the AOCS Official Method Ba 3-38 (AOCS, 2001)).

For the FA analysis, lipids were extracted from the samples using the cold extraction process, which involves mixing/homogenizing with chloroform: methanol solution (Karlovíč and Andrič, 1996; Ivanov et al., 2012). The samples obtained were analyzed using the Gas Chromatographer Agilent 7890A system (Agilent Technologies, CA, USA) with a Flame Ionization Detector (GC-FID), equipped with fused silica capillary column (Supelco SP®-2560 Capillary GC Column) and helium as a carrier gas. The FAs peaks were identified by the comparison of retention times with retention times of standards from the Supelco 37 component FA methyl ester mix and with the data from internal data library, based on previous experiments and FA methyl ester determination using a GC-Mass Spectrometer.

After extrusion, the feed compound was dried and stored in two different manners: under ambient conditions (24 ± 2 °C) for three weeks and the in a climate chamber under accelerated conditions of high temperature (65 °C) and air humidity (φ = 70 %) for two months. The following parameters were successively measured during the period of storage: the microbial status of the samples, the peroxide value as a measurement of primary oxidation products, as well as the BARs value and anisidine value (AV) as measurements of secondary oxidation products. The AV value indicates the secondary oxidation of oil or fat, which is mainly imputable to aldehydes and ketones.

The peroxide value was measured in the extracted fat phase of the co-extrudate according to the AOCS Official Method Cd 8-53 (Firestone, 1989). The cold extraction of the fat phase was performed with isooctane. The values were expressed as mmol H2O2 per kg of fat phase extracted from the sample. The AV analysis was done as proposed by the AOCS’s official method CD 18-90 (AOCS, 2017). TBARS determination was done according to the modified spectrophotometric method (Voljić et al., 2011).

All of the results were expressed as means. The standard deviation of the means obtained (SD) was calculated using Microsoft Excel 2010 (Microsoft; Redmond, USA). Statistical analyses of the experimental data were performed using STATISTICA 13 (Statsoft, Inc., 2015). The analysis of variance (ANOVA) and the Tukey HSD test for comparison of sample means were used to analyze variations. The level of confidence was set at 95 %.

RESULTS AND DISCUSSION

The results of the chemical analyses of produced black mustard seed (BMS) and white mustard seed (WMS) extrudates are shown in Table 1.

Table 1. Chemical composition of extruded black mustard seed (BMS) and white mustard seed (WMS)

| Quality parameters | Extruded BMS | Extruded WMS |
|--------------------|--------------|--------------|
| Moisture content (%) | 5.51 ± 0.21 | 4.96 ± 0.14 |
| Crude protein content (%) | 31.24 ± 0.92 | 28.01 ± 1.05 |
| Crude fat (%) | 19.12 ± 1.01 | 26.41 ± 2.01 |
| Ash content (%) | 5.15 ± 0.63 | 5.02 ± 0.41 |
| Crude fibre content (%) | 24.40 ± 1.41 | 23.21 ± 1.12 |

The results are presented as mean ± standard deviation, n=3.

According to the chemical composition, both extrudates were highly rich in crude protein (31.24 % in BMS and 28.01 % in WMS extrudate) and fat content (19.12 % in BMS and 28.01 % in WMS extrudate). These results indicate that the extrudates produced have a great potential to be used as protein, as well as energy sources in the feed production. Provided the results obtained are compared with other feedstuff protein and fat contents, it can be concluded that the extrudates produced are similar to extruded soybean (Sauvant et al., 2004). However, high crude fiber contents exceeding 20 % cannot be disregarded. Dietary fibers have an important role in pig and poultry diets as they maintain the normal physiological function in the digestive...
Nevertheless, a high dietary fiber content is associated with decreased nutrient utilization and low net energy values (Lindberg, 2014). Table 2 presents the fatty acid (FA) analysis of both extrudates.

Table 2. Fatty acid composition of the extruded BMS and WMS

| FA content (% of total FAs) | BMS | WMS |
|-----------------------------|-----|-----|
| Not extruded                |     |     |
| SFA                         | 4.70 ± 0.83 | 5.78 ± 1.01 |
| MUFA                        | 54.37 ± 2.01 | 54.91 ± 1.01 |
| PUFA                        | 40.92 ± 2.14 | 39.31 ± 1.01 |
| UFA                         | 95.30 | 94.22 |
| Extruded                    |     |     |
| SFA                         | 3.62 ± 1.01 | 4.45 ± 1.01 |
| MUFA                        | 63.64 ± 1.01 | 62.84 ± 1.01 |
| PUFA                        | 32.74 ± 1.01 | 32.71 ± 1.01 |
| UFA                         | 96.38 | 95.55 |

SFA – saturated fatty acid, MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid

The results are presented as mean ± standard deviation, n=3.

According to the results obtained, the WMS extrudate is richer in polyunsaturated fatty acid (PUFA) content than the BMS extrudate. The extrusion process caused a decrease in the PUFA content in both seed extrudates. Since both extrudates had extremely high contents of unsaturated fatty acid (UFA) (above 90 %), it is reasonable to expect that these products are highly susceptible to oxidation. It must be emphasized that both the BMS and WMS extrudates contained a large share of erucic acid (EA), the FA well known as an antinutrient. The EA content in the BMS extrudate was 34.21 %, whereas the EA content was 43.17 % of the total FAs in the WMS extrudate. The extrusion process caused a decrease in the PUFA content in both seeds. However, decreases in the content of EA were statistically insignificant (p < 0.05). Natural forms of mustard species contain high levels of EA, usually even more than 40 % of the total FAs (Sissener, et al., 2018). The heart is mainly influenced by toxic effects following short-term or long-term exposure, which was confirmed in several experiments on rats, pigs, monkeys, rabbits, etc. The most common and sensitive effect observed in all species is myocardial lipidosis. Studies involving rats and pigs showed a relationship between the level of EA in the diet and the severity of myocardial lipidosis. The overall no-observed-adverse-effect level for lipidosis was 0.7 g/kg of body mass per day in a 2-week feeding study in newborn piglets. Adult pigs are able to tolerate higher levels of EA than young animals. The lowest observed-adverse-effect level of EA for liver toxicity in poultry was 0.02 g/kg of body mass per day. Therefore, special attention has to be paid when feeding animals with both mustard seed by-products (EFSA, 2016).

Figure 1 shows the PV of the extrudates produced under accelerated conditions. The maximum PV of the extruded WMS was 5.79 mmol/kg of fat phase, whereas the PV of the extruded BMS did not exceed 0.44 mmol/kg of fat phase, and it remained quite stable. The PV of the extruded WMS and BMS stored under ambient conditions for two months amounted to 0.26mmol/kg and 14.40 mmol/kg, respectively.

The WMS by-product also showed significantly higher (p < 0.05) values of the AV then those recorded in the BMS by-product when stored in a climatic chamber (Figure 2). The AV of the samples stored for two months under ambient conditions amounted to 6.50 % in the WMS and 0.40 % in the BMS extrudate (Figure 3).

The TBARS values increased significantly faster (p < 0.05) in the WMS by-product than in the BMS by-product (Table 3). Both parameters of the primary and secondary oxidation processes indicated that the extruded by-products of BMS exhibited better oxidative stability than the by-product of WMS. This can be explained by a higher PUFA content in the WMS extrudate. Furthermore, the antioxidant potential of both seeds should be investigated.
as their controlled dosing and planned inclusion in animal diets. The separation of oil and the use of mustard seed meals, as well especially a high content of EA. One of the solutions could be animal nutrition such as a relatively high fiber content and there are great impediments to their undisturbed application in of their high protein contents and high fat contents. However, great potential as animal feed compounds, especially on account

| Ambient conditions | Climatic chamber |
|--------------------|------------------|
| **TBARS concentration (nmol/ g)** | **TBARS concentration (nmol/ g)** |
| Storage | BMS | WMS | Storage | BMS | WMS |
| Day 1 | 35.31 | 32.14 | Day 3 | 39.12 | 33.25 |
| Day 5 | 36.12 | 33.18 | Day 6 | 44.15 | 49.14 |
| Day 10 | 38.96 | 35.01 | Day 9 | 56.78 | 60.27 |
| Day 15 | 41.15 | 40.74 | Day 12 | 74.12 | 81.68 |
| Day 20 | 49.64 | 53.16 | Day 15 | 90.41 | 101.47 |
| Day 25 | 53.87 | 56.48 | Day 18 | 128.47 | 144.08 |
| Day 30 | 65.01 | 69.15 | Day 21 | 145.01 | 160.22 |
| Day 35 | 76.74 | 77.46 | Day 30 | 98.14 | 103.64 |
| Day 40 | 83.44 | 86.54 | Day 25 | 105.21 | 106.14 |
| Day 45 | 91.32 | 95.19 | Day 20 | 103.17 | 111.05 |
| Day 50 | 98.14 | 103.64 | Day 15 | 98.14 | 103.64 |
| Day 55 | 105.21 | 106.14 | Day 10 | 91.32 | 95.19 |
| Day 60 | 103.17 | 111.05 | Day 5 | 39.12 | 33.25 |

The results are presented as means of three replicate

**CONCLUSIONS**

The extruded by-products of BMS and WMS have shown a great potential as animal feed compounds, especially on account of their high protein contents and high fat contents. However, there are great impediments to their undisturbed application in animal nutrition such as a relatively high fiber content and especially a high content of EA. One of the solutions could be the separation of oil and the use of mustard seed meals, as well as their controlled dosing and planned inclusion in animal diets.

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**REFERENCES**

Abul-Fadl, M.M., El-Badry, N., Ammar. M.S. (2011). Nutritional and chemical evaluation for two different varieties of mustard seeds. World Applied Sciences Journal, 15 (9), 1225-1233.

Becker, G. (2008) Livestock feed costs: Concerns and options; Washington, DC.

Commission Regulations 442/1975/EEC, 1975. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1975L0442:20031120:EN:PDF

EFSA Panel on Contaminants in the Food Chain (CONTAM) (2016). Erucic acid in feed and food. EFSA Journal, 14 (11), 4593-4766.

Firestone D. (1989). Official Methods and Recommended Practices of the American Oil Chemists’ Society. 4th Edition. American Oil Chemists’ Society, Champaign, IL.

Food waste management in the United States, 2014, Environmental Protection Agency EPA, (2016). Office of Resource Conversation and Recovery, 2-18.

Table 3. TBARS concentration in the extruded BMS and WMS during storage under ambient conditions and in a climatic chamber

Folch, J., Lees, M., Sloane, S. G.H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry, 226(1), 497-509.

Galanakis, C. (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. Trends in Food Science and Technology, 26, 68–87.

Gustavsson, J., Cederberg, C., Sonesson, U., Emanuelsen, A. T. (2013). The methodology of the FAO study: “Global Food Losses and Food Waste - extent, causes and prevention”, pp 2-70.

Ivanov, D., Čolovic, R., Lević, J., Sredanović, S. (2012). Optimization of supercritical fluid extraction of linseed oil using RSM. European Journal of Lipid Science and Technology, 114 (7), 807–815.

Karlović, D., Andrić, N. (1996). Kontrola kvaliteta semena uljarica. Savezni zavod za standardizaciju, Tehnološki fakultet Novi Sad.

Kornmanjoš, Š., Rakita, S., Spasevski, N., Marjanović Jeromela, A., Popović, S., Banjac, V. (2017). Quality of by-products from white (Sinapis Alba) and black (Brassica Nigra) mustard seed processing. Fifth International Conference Sustainable Postharvest and Food Technologies INOPTEP 2017, National Society of Processing and Energy in Agriculture, Vršac, Republic of Serbia.

Lindberg, J.E. (2014). Fiber effects in nutrition and gut health in pigs. Journal of Animal Science and Biotechnology, 5, 15-22.

Official Methods of Analysis, Association of Official Agricultural Chemists (AOAC) (2000). 17th AOAC International, Arlington, VA, USA.

Official Methods and Recommended Practices, American Oil Chemists Society (AOCS) (2001). Method Ba 3-38 ‘. (Ed.): Firestone, D. 5th Edition, AOCS, Champaign, IL, USA.

Official Method, American Oil Chemists Society AOCS Cd 18-90, Reapproved (2017). Champaign, IL, USA.

Sauvant, D., Perez, J.-M., Tran, G. (2004). Tables of composition and nutritional value of feed materials, Wageningen Academic Publishers, Wageningen (the Netherlands) and INRA, Paris (France).

Sissener, N.H., Ørsrud, R., Sanden, M., Froyland, L., Remo, S., Lundebye, A.K. (2018). Erucic acid (22:1n-9) in fish feed, farmed, and wild fish and seafood products. Nutrients, 10, 1443-1463.

Voljić, M., Frankić, T., Levat, A., Nemec, M., Salobir, J. (2011). Evaluation of different vitamin E recommendations and bioactivity of α-tocopherol isomers in broiler nutrition by measuring oxidative stress in vivo and the oxidative stability of meat. Poultry Science, 90, 1478–1488.

Vukmirović, Đ., Čolović, R., Ivanov, D., Kokić, B., Lević, J., Đuragić, O., Sredanović, S. (2011). Uticaj nezavisnih parametara ekstrudiranja na temperaturni profil u cevi ekstrudera. Journal on Processing and Energy in Agriculture, 15(2), 94-97.

Wanasundara, J. (2008). Mustard as an ingredient in food processing: Current uses and the potential. In: Mustard Grower, 3–5. Canada.

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