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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" ogleda se u što masovnijoj upotrebi u daljim ciklusima prerade. S obzirom na samo poreklo sirovina, kao najlogičniji i najjednostavniji vid dalje upotrebe odbačenih semenskih nusproizvoda nameće se proizvodnja hrane za životinje. Stoga je cilj ovog istraživanja bio da se ispita mogućnost upotrebe nusproizvoda semenske dorade crne i bele slačice u proizvodnji novog hraniva za ishranu životinja uz primenu tehnološkog postupka ekstrudiranja. Imajući u vidu hemijski sastav sirovine, nakon ekstrudiranja pristupljeno je ispitivanju je skladišne stabilnosti proizvedenih ekstrudata, praćenjem oksidativnih promena masne faze proizvoda. Proizvedeno hranivo je osušeno i skladišteno na dva načina. Prva grupa uzoraka skladištena je na sobnim uslovima temperature i vlažnosti vazduha, dok je druga grupa uzoraka skladištena u klima komori pri ubrzanim uslovima povišene temterature (65°C) i vlažnosti vazduha (φ = 70%). Sledeći parametri sukcesivno su praćeni tokom perioda skladištenja: mikrobiološki status uzoraka, peroksidni broj kao merilo primarne oksidacije proizvoda i supstance reaktivna sa tiobarbiturinskom kiselinom (Thiobarbituric Acid Reactive Substances – TBARS) i anisidinski broj kao merilo sekundarne oksidacije proizvoda. Dobijeni rezultati ukazuju na to da proizvedeni ekstrudati pokazuju značajan potencijal kao novo hranivo za životinje. Visok sadržaj proteina, a u isto vreme i masti, svrstavaju ekstrudirane nusproizvode slačice u proteinsko-energetska hraniva. Poredeći ova dva parametra sa vrednostima konvencionalnih hraniva, može se reći da su oba ekstrudata slična ekstrudiranoj soji. Međutim, ono što predstavlja problem, jeste visok sadržaj celuloze (preko 20% u oba ekstrudata), što je značajan limitirajući faktor u ishrani životinja. Za proizvedene ekstrudate karakterističan je visok sadržaj nezasićenih masnih kiselina. Ograničenje u nesmetanoj upotrebi ovih ekstrudata u ishrani životinja predstavlja visok sadržaj eruka kiseline, jedinjenu poznatom po svom antinutritivnom dejstvu, a koji ekstrudiranjem nije bilo moguće smanjiti. Takođe je utvrđeno da je ekstrudat proizveden od nusproizvoda crne slačice stabilniji od ekstrudiranih nusproizvoda bele slačice.

***Ključne reči**: Crna slačica, Bela slačica, nusproizvodi, ekstrudiranje, 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 (*Sinapsis 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 (*Kormanjoš 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 gravimetrical 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 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 mixture (2:1) according to the method by *Folch et al. (1957)*. FA methyl esters were prepared from the extracted lipids by the transmetilation method using a 14 % wt boron trifluoride/methanol solution (*Karlović and Andrić, 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 H_2O_2 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 (*Voljč 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 tract. 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	Extruded	Not extruded	Extruded
SFA	4.70 ± 0.83	5.78 ± 1.01	3.62 ± 1.01	4.45 ± 1.01
MUFA	54.37 ± 2.01	54.91 ± 1.01	63.64 ± 1.01	62.84 ± 1.01
PUFA	40.92 ± 2.14	39.31 ± 1.01	32.74 ± 1.01	32.71 ± 1.01
UFA	95.30	94.22	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*).

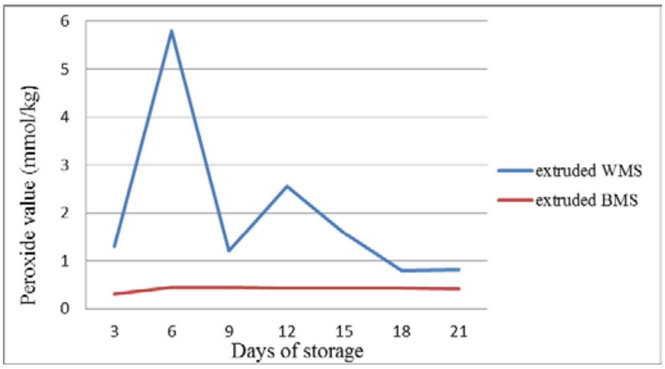

Fig. 1. Peroxide values of the extruded BMS and WMS during storage in a climatic chamber

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.

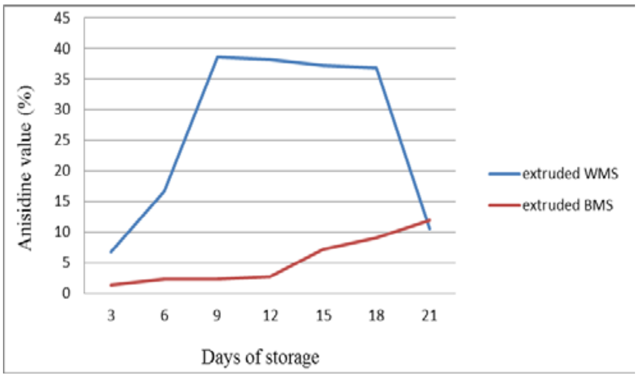

Fig. 2. Anisidine values of the extruded BMS and WMS during storage in a climatic chamber

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).

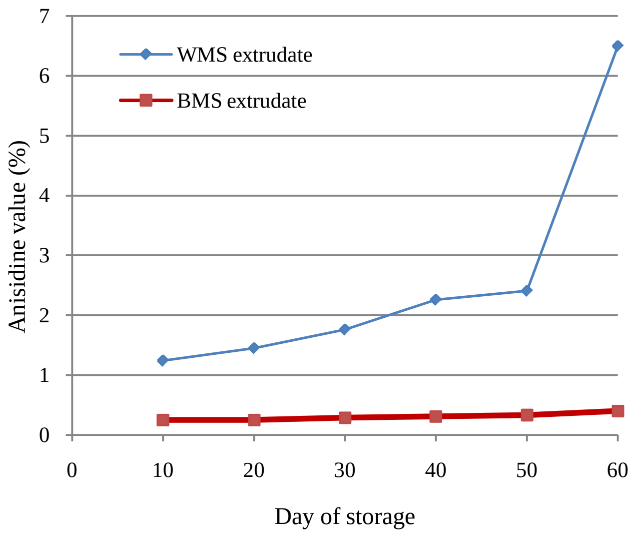

Fig. 3. Anisidine values of the extruded BMS and WMS during storage under ambient conditions

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.

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

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

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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