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Impact of Preconditioner Moisture on the Microstructure of the Nile tilapia Extrudates Including High Sunflower Meal Inclusion

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ABSTRACT

The inclusion of sunflower meal to substitute soybean meal protein was examined for its effect on the physical properties and microstructure of extrudates processed under two different preconditioner moistures. Two diets were formulated by substituting soybean meal with sunflower meal at levels of 0 and 50%. The two diets were processed under two preconditioner moisture levels of 15 and 20% to produce four different extrudates. Interaction between preconditioner moisture and the substitution factors showed a highly significant effect on expansion percentage, bulk density and apparent porosity % of the extrudates, while no significant effect was detected on maximum oil absorption and fat leakage. The extrudates of sunflower meal appear less expanded, with less diameter and apparently darker than soybean meal extrudates. The surface morphology of sunflower meal pellets extruded with high preconditioner moisture was more compact and less cracked with fewer pores. It seems that the cellulose of the extrudates increased with more inclusion of sunflower meal, affecting the physical probability and microstructure.

INTRODUCTION

The global expansion in the aquaculture sector resulted in high demand for fish meals that are considered the golden raw material for the aquatic feed. Fish meal has been considered unsustainable feedstuff, and thus more sustainable practices in the aquaculture industry are required. In this respect, aqua feed must be developed to produce more efficient feed with less fish meal inclusion. Some plant protein sources are considered a good and moderate alternative sources with lower prices for fish meal such as soybean meal, rapeseed meal, cottonseed meal and sunflower meal. With increasing the competition between human food and animal for soybean meal **(Gatrell *et al.*, 2014)** and prices elevation, alternative plant protein sources at low prices and high quality should be scrutinized. Due to its high content of digestible protein, amino acids content and the absence of anti-nutritional factors **(Sanz *et al.*, 1994; Knudsen, 1997; Senkoylu & Dale,**

1999), sunflower meal stands as a promising plant protein source. Beside feed formula, the development of processing conditions is highly required to maximize the nutritional value of feed regarding fish requirements and behavior. In addition to using a high nutritional plant protein source in aquafeed, the physical characteristics must be adequate since raw material has a great effect on those characteristics **(Draganovic *et al.*, 2011)**. Plant protein sources and their concentration may affect the mechanical behavior of the extrudates melt, changing the microstructure of the extrudates as shown for wheat gluten in the study of **Draganovic *et al.* (2013)**. Remarkably, sunflower meal contains higher levels of cellulose, xylose and non-starch polysaccharide (NSP) than those of soybean meals **(Irish & Balnave, 1993)**. In this context, insoluble high fiber level could restrict air developing, decreasing subsequently the expansion ratio **(Alam *et al.*, 2014)**. The nature and type of ingredients in addition to the extrusion parameters change the expansion extent **(Singh & Muthukumarappan, 2014)**.

Expansion is one of the most important physical parameters which may affect several other physical parameters as unit density, bulk density, apparent porosity, oil absorption and fat leakage. On the other hand, the moisture content of a mixture seems to affect the expansion ratio as reported in the study of **Singh and Muthukumarappan (2014)**. In their study, they reported that the elevation of the mixture moisture increased the expansion ration up to a limit, while more moisture inclusion could negatively affect the ratio.

Thus, the objective of the study was to examine the probable extent of the moisture's percentage in the preconditioner needed for improving the physical parameters of the extrudates including sunflower meal as a substitution for soybean meal.

MATERIALS AND METHODS

Experimental design

This experiment was conducted using two levels of two independent variables; i) preconditioner moisture (factor M) at (15 and 20%) ii) substitution of soybean meal protein with sunflower meal protein (factor S) at (0 and 50%).

Feed blends formulation

Two experimental diets (30% crude protein; 386.03Kcal/100g) were formulated as diet (I) represented the control(SBM), where the main protein source was soybean meal (43.7% soybean meal, 0% sunflower meal). Protein of sunflower meal replaced 50% of soybean meal protein in diet (II) (SFM50; 22% soybean meal, 29.2% sunflower) Table (1).

Table 1. Ingredient composition of feed blends and proximate chemical composition of blends used in the study (% dry matter)

Feed ingredient	**Mass of ingredient (% DM)**	
	Blend I	Blend II
Soybean meal 46%	43.7	22
Sun flower meal 36%	0	29.2
Yellow corn	20	20
Wheat bran	18	10.07
DDGS	3	3
Corn Gluten meal 60%	1	1
Poultry offal meal	6	6
Fish oil	1	1
Limestone	2.5	2.5
Salts	1	1
Mono- calcium phosphate	1	1
Vitamins and mineral mix	2.8	3.23
Chemical composition	Mass of ingredient (% DM)	
Dry matter	90.02	92.17
Moisture	9.98	7.83
Crude protein	30.3	29.77
Crude fat	1.65	1.85
Crude ash	9.35	9.15
Crude fiber	4.3	8.6
NSP*	17.76	19.83
NFE**	44.42	42.8
Gross energy ($Kcalkg^{-1}$)***	3689.69	3611.91

* NSP; non starch polysaccharides was calculated as concentration in each of the feed ingredients were sited from **Kocher *et al.* (2000)**, **Knudsen (2014)** and **Jaworski *et al.* (2015)**

** NFE by difference; Nitrogen Free Extract = 100 – (crude protein + crude fat + crude fiber + moisture + ash)

***Dietary gross energy was calculated using the conversion factors of 5.65, 9.45 and 4.1 kcal/g for protein, lipids and NFE, respectively **(Hepher *et al.*, 1983)**.

The ingredients were mixed and processed in Cairo feed company (Fish feed plant, Nubariyah, El Beheira Governorate, Egypt). Table (2) represents the values of NSP content of each feed ingredient and feed formula. Regarding the calculation, the two diets had nearly the same total NSP value. However, the soluble NSP was 13.34 and 7.37 % for SBM diet and SFM50 diet, respectively. The two diets extruded under two different preconditioner moisture levels (15% and 20%), producing four different extrudates.

Extrusion

The extrudates were processed using a commercial single-screw extruder (Sprout Matador EX620 Netherland, 250KW, 335 HP), with the die of 3mm. The extruder barrel consisted of five head sections; the cooling water lines were two separated lines, and the first one was for the first section only. On the other hand, the second line was for the other sections (sections 2–5). Barrel conditions (screw speed and barrel cooling flow) were fixed during processing for all experimental diets to study the effect of preconditioner moisture for the extrudates' physical characteristics and micro structure. Additionally, the preconditioner temperature was fixed for all experimental diets (95°C), monitored by an external digital reader. the preconditioner moisture was controlled by manual water valve. Andritz (type BT 2x8) commercial horizontal air dryer was used for drying the extrudates. After drying, oil editing was conducted by Sprout matador sprayer coating system without air vacuum. At the end of the processing line, a swivel valve cooler (Geelen counterflow) was used for cooling the extrudates to ambient room temperature, followed by packing (25kg).

Table 2. Non-starch polysaccharide (NSP) composition of soybean meal and sunflower meal (% dry matter)

	Soybean meal	Sunflower meal	Yellow corn	Wheat bran	DDGS	Corn gluten meal 60%	SBM	SFM50
Cellulose	6.2	12.4*	1.7	6.4	5.8	0.9	4.38	6.19
Arabinose	2.6	3.44	1.7	7.7	5.2	0.6	3.02	2.90
Xylose	1.9	6.18	2.1	14.4	7.1	0.7	4.06	4.40
Mannose	1.3	1.57	0.2	0.5	1.9	0.3	0.76	0.90
Galactose	4.1	2.25	0.8	0.8	1.3	0.3	2.13	1.85
Glucose	0.7	14.11	0.6	3.4	2.1	0.5	1.10	4.83
Total NSP	21.7	31.2	8.1	34.5	25	3.6	18.06	19.98

*****In the whole sunflower meal as reported by **Knudsen (2014)**
Data provided from previous studies **(Düsterhöft *et al.*, 1991; Kocher *et al.*, 2000; Knudsen 1997; Jaworski, *et al.*, 2015)**
NSP: Non-starch polysaccharides; NCP: Non-cellulosic polysaccharides.

All extrudates samples of different diets were collected from commercial feed pack at aquaculture research unit and stored at the ambient lab temperature till chemical and physical analyses and SEM were performed. All the extrudates were chemically analyzed in the Carat laboratory – Adisseo France (Commentry, France). According to **AOAC (2016)**, the average chemical composition of the two extrudates of each diet was calculated, and data are presented in Table (1).

Physical properties

Bulk density

Bulk density (BD) was determined as a ratio of the mass of extrudates that they filled up to a given bulk volume and was measured using a plastic cup (1 kg of water) following the procedures of **Chevanan *et al.* (2008)**.

The apparent porosity

The apparent porosity (App %) of a given extrudate sample was calculated as APP = (1- bulk density/unit density (UD)) *100 according to the method of **Chevanan *et al.* (2008)**. Unit density (UD; g/cm3) was calculated as the ratio of the mass of each extrudate pellet to the calculated volume of that piece (assuming cylindrical shapes for each extrudate sample).

Maximum oil absorption

Samples of the extrudates (100 g) from each treatment were submerged in excess (80g) heated (60 ℃) fish oil, placed in plastic cups and then in a custom made vacuum device (in lab made glass box 0.8mm, with a vacuum pump of 750kw). After arranging all the sample cups inside the vacuum chamber, the vacuum pump was turned on, and the air pressure inside the chamber was slowly decreased. The air pressure was around -0.3 bar for 1–1.5min inside the vacuum chamber. When the air bubbles escaped from the extrudates, and the visible signs showed that it was ceased, the vacuum pump was turned off, and the chamber was re-equilibrated to the atmospheric pressure of the lab room. Afterwards, all the cups were removed from the chamber, and any excess oil was removed via pouring and then using absorbent paper towels to be sure that excess oil no more exists. The relative oil uptake by the extrudates (maximum oil absorption) was calculated as difference between the final weight of the extrudates infused with oil and the extrudates sample weight (100g).

Fat leakage analysis

From the previous analysis (maximum oil absorption), all samples were placed on blotting paper in a plastic dish at 40°C for 24h. The fat leakage was calculated as the loss weight of fat from each previous sample of the extrudates.

Scanning electron microscopy (SEM)

Extrudate samples were coated with a thin layer of gold by ion sputtering and examined using a JEOLJSM-5200 scanning electron microscope (Tokyo, Japan) in nematode laboratory, Faculty of Agriculture Farm, Cairo University, using the method of **Vehof *et al.* (2001)**.

Statistical analysis

The data are presented in the Tables as means with a mean standard error. Physical parameters of the extrudates were analyzed using one-way ANOVA to test the effect of the factors preconditioner moisture, (M) and substitution, (S) using Jeffreys's Amazing Statistics Program (JASP). Means were compared by the Tukey's range test.

RESULTS

Interaction between preconditioner moisture and the substitution factors showed high significant effect on expansion percentage, bulk density and apparent porosity% of the extrudates, while no significant effect on maximum oil absorption and fat leakage was observed. With increasing inclusion levels of sunflower meal, a significant increase in the bulk density and an apparent porosity was observed (Table 3).

Table 3. Effect of preconditioner moisture and substitution of soybean meal with sunflower meal on bulk density, apparent porosity%, maximum oil absorption and leakage

		Expansion percentage %	Bulk density	Apparent porosity%	Maximum oil absorption	Leakage
Interaction						
SBM	**LM**	39.63±0.904^{a}	466.16±2.519^{bc}	27.3±0.407^{c}	١٢١.٥٢±0.989	6.44±0.508
	HM	36.79±0.904^{ab}	472.14±2.519^{b}	19.64±0.407^{d}	١٢٠.٥±0.989	5.79±0.508
SFM50	**LM**	34.53±0.904^{b}	456.92±2.519^{c}	41.12±0.407^{a}	١٢٤.٣٧±0.989	4.26±0.508
	HM	27.08±0.904^{c}	492.56±2.519^{a}	36.69±0.407^{b}	١٢٠.٩٦±0.989	4.14±0.508

In each column, data having different superscripts are significantly different (P<0.05).
Data are means ± SEM.
SBM: Soybean meal diet; SFM50: Sunflower meal diet; LM: Low moisture; HM: High moisture; S: Substitution; M: Moisture.

Stereomicroscopy images support the expansion% results of different treatment Figs. (1&2).

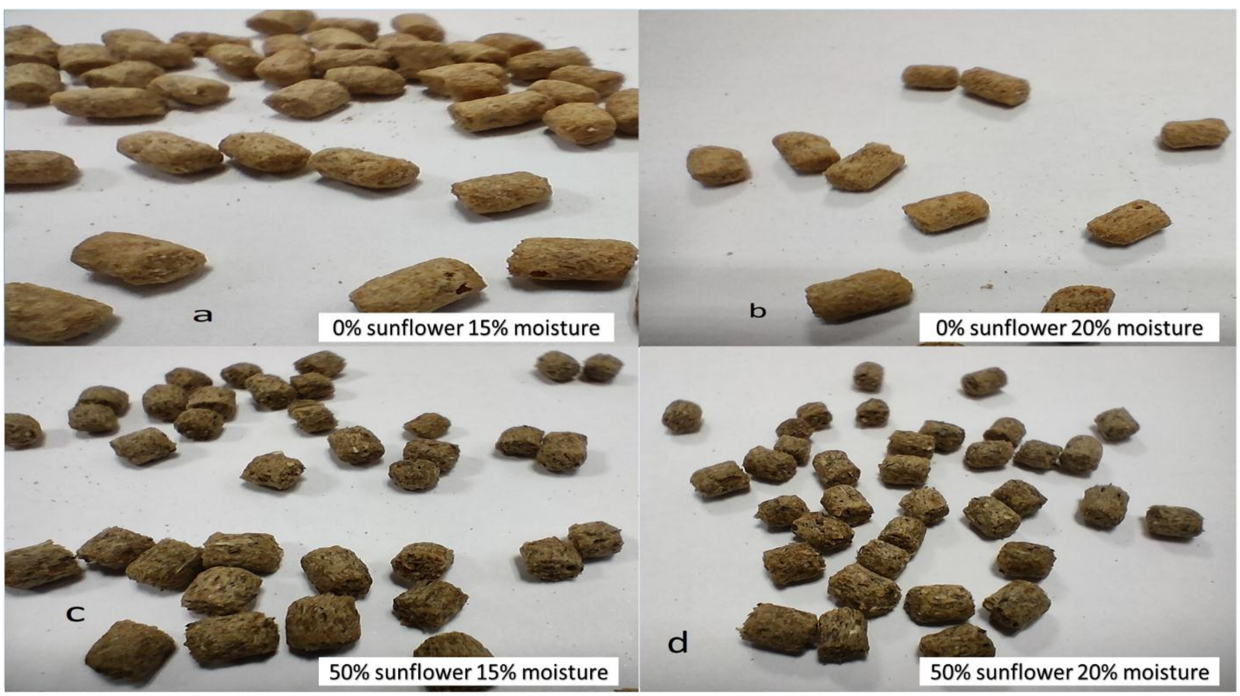

Fig. 1. Extrudates morphology for feed with and without sunflower meal

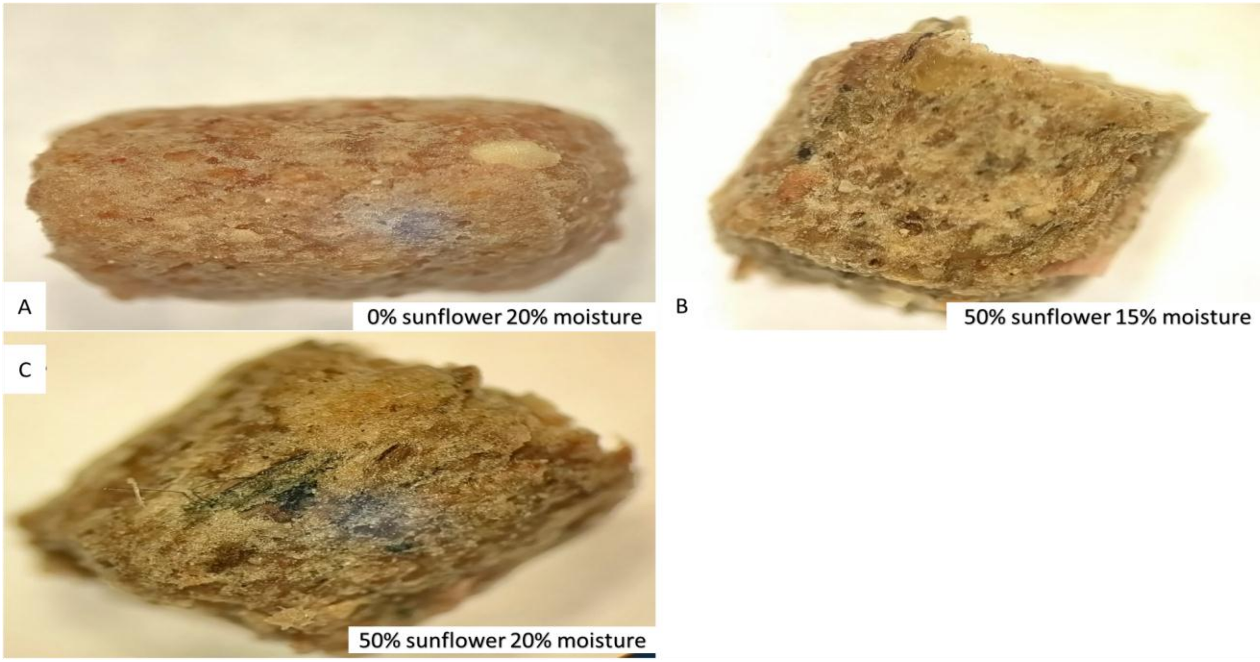

Fig. 2. Extrudates morphology for the feed with and without sunflower meal

The extrudates of sunflower meal appear less expanded, with a less diameter and apparently darker than soybean meal extrudates. The surface morphology of sunflower meal pellets extruded with high preconditioner moisture was more compact and less cracked with less pores (Fig 3).

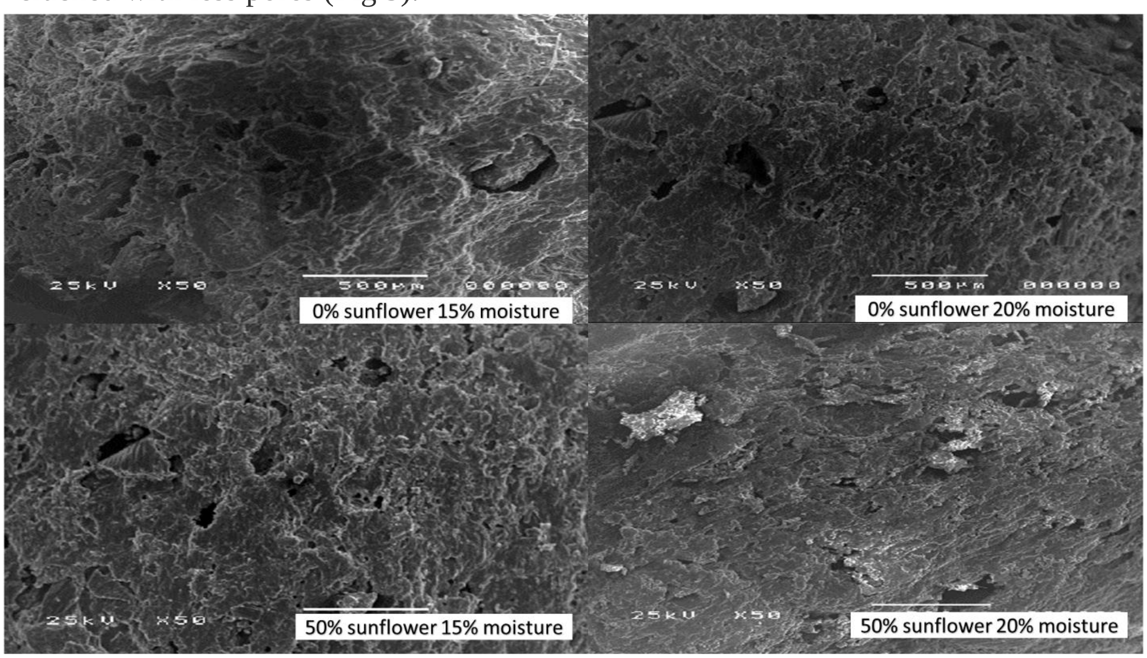

Fig. 3. Scanning electron micrographs of the outer surface of extrudates (magnification X200).

These compact extrudates represented the result of less expansion% and higher bulk density in the final product of the sunflower meal. The cross section of the extrudates (50X and 200X, fig 4&5) showed the same trend of the outer surface morphology since high sunflower meal-formula extruded in the presence of high preconditioner moisture showed more compact structure and less cracks. On the other hand, soybean meal extrudates were characterized by a large number of small pores, with uneven size in the inside.

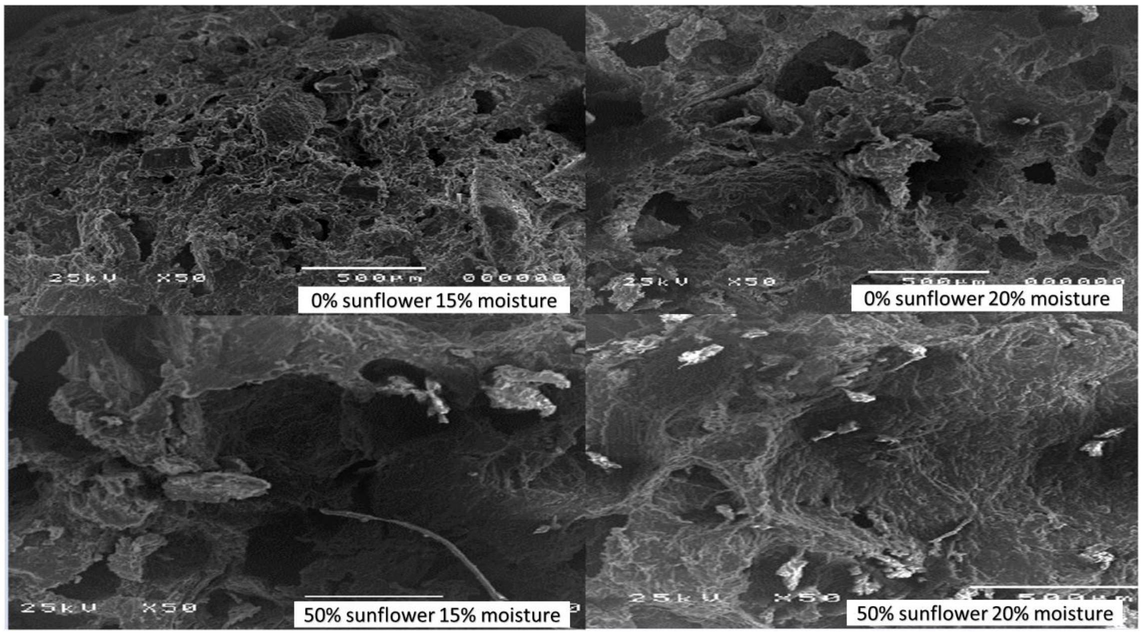

Fig. 4. Scanning electron micrographs of the extrudates cross section (magnification X50)

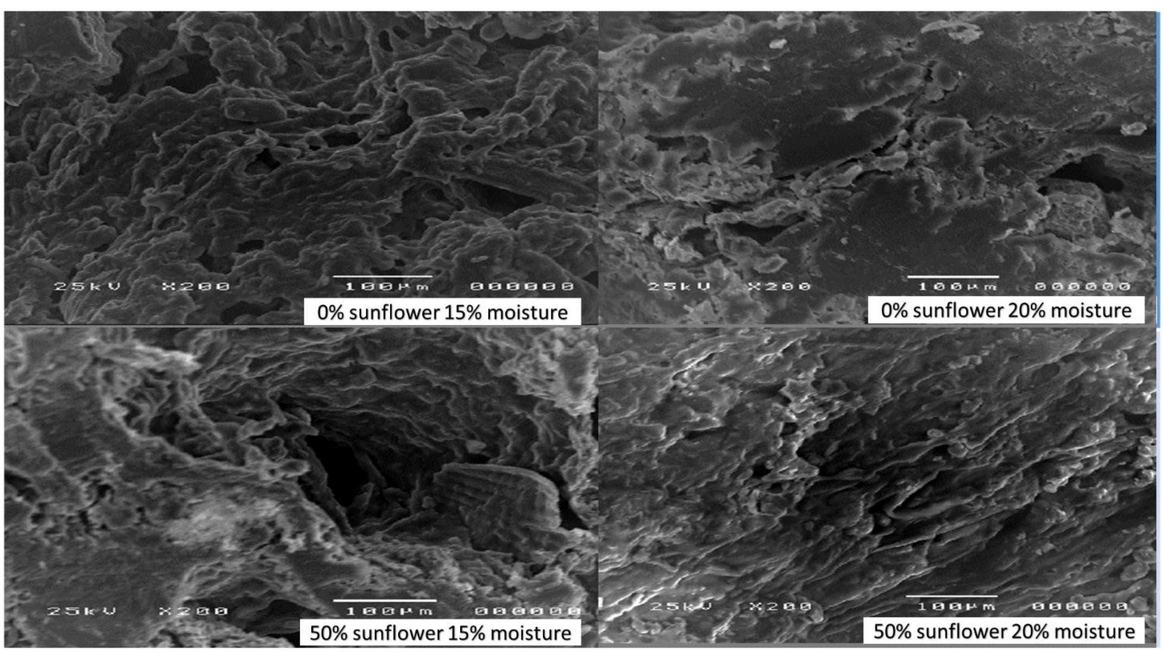

Fig. 5. Scanning electron micrographs of the extrudates cross section (magnification X200)

DISCUSSION

The nutritional values of dietary raw materials are not the only factor that needed to be met while partial or complete substitution of different feedstuffs, the required physical properties must also be achieved. Preconditioning and extrusion condition cause significant changes for raw materials (**Sørensen, 2012**). The results showed that high inclusion of sunflower meal decreases the expansion ratio significantly, and that could be correlated to the high non- soluble NSP in sunflower meal. It was suggested that feed ingredients, fiber levels, fiber particle size, NSP and extrusion conditions had effects on the expansion ratio (**Chevanan *et al.*, 2007; Glencross *et al.*, 2010; Alam *et al.*, 2014; korkerd *et al.*, 2016: monti *et al.*, 2016; Kallu *et al.*, 2017**). Moreover, the previous authors suggested that fiber levels, source and particle size are related to the increase in the disruption of air cell development and rupture of the cell wall occur and subsequently, restricting air developing effect on the extrudates cell decreasing the expansion ratio. In this context, **Kraugerud *et al.* (2011)** reported that, adding purified starch showed a significant correlation with the expansion rate ($r = 0.405$; $P<0.001$), while the amount of cellulosic NSP was negatively correlated to the expansion ($r = -0.603$; $P<0.001$).

Stereomicroscopy images and SEM micrographs are impressive tools that could be used as indicator for the extrudates structural changes. It was noticed that, sunflower meal showed an effect on the extrudates color, surface morphology and microstructure compared to soybean meal.

SEM micrographs indicate that dietary inclusion of sunflower caused less pores with larger size than soybean meal, whereas more water inclusion in the preconditioner upon processing sunflower meal –diets resulted in pellets with more compact surface. The physical changes in the extrudates microstructure might be due to the different mechanical behavior of the melt substance during extrusion, regarding the chemical composition of the formula. Fiber content of the formula seems to be one of the major components that may affect the physical parameters in the current study. Microstructure of high fiber-sunflower meal extrudates showed unevenly- sized large pores in the inside, while extrudates of low fiber-sunflower meal showed uniform pores and higher expansion ratio **(Banjac *et al.*, 2021).** Supplementation of high cellulose level of 10% caused a cell size reduction of the extruded cassava starch since it might cause fiber accumulation and non-uniform distribution of fiber in the starch matrix **(Kaisangsri *et al.*, 2019)**. Meanwhile, high fiber level may increase the roughness of pellet surfaces as shown with more dietary inclusion of rapeseed press cake or rapeseed peel in the study of **Martin *et al.* (2019)**.

CONCLUSION

Each plant protein source has different effect on the mechanical behavior of the melt during extrusion. This difference was obvious in the distribution of air cells and the extent of expansion. More inclusion of sunflower meal in the formula resulted in darker color of the extrudates, less pores on the pellet surfaces and large pores in the inside. Moisture elevation resulted in more internal and external compacted structural of the sunflower meal extrudates. Sunflower meal could be included in fish diets up to 30%, where extrusion condition could improve the physical properties of the extrudates.

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REFERENCES

Alam, S.A.; Järvinen, J.; Kirjoranta, S.; Jouppila, K.; Poutanen, K. and Sozer, N. (2014). Influence of particle size reduction on structural and mechanical properties of extruded rye bran. Food and Bioprocess Technology, 7(7): 2121-2133.

Association of Official Analytical Chemists (AOAC). (2016). Official method of analysis, 2016 20th edition, 973.18. chapter 4, pages online.

Banjac, V.; Vukmirović, Đ.; Pezo, L.; Draganovic, V.; Đuragić, O. and Čolović, R. (2021). Impact of variability in protein content of sunflower meal on the extrusion process and physical quality of the extruded salmonid feed. Journal of Food Process Engineering, 44(3): p.e13640.

Chevanan, N.; Muthukumarappan, K.; Rosentrater, K.A. and Julson, J.L. (2007). Effect of die dimensions on extrusion processing parameters and properties of DDGS- based aquaculture feeds. Cereal chemistry, 84(4): 389-398.

Chevanan, N.; Rosentrater, K.A. and Muthukumarappan, K. (2008). Effect of DDGS, moisture content, and screw speed on physical properties of extrudates in single- screw extrusion. Cereal Chemistry, 85(2):132-139.

Draganovic, V.; Van der Goot, A.J.; Boom, R. and Jonkers, J. (2013). Wheat gluten in extruded fish feed: effects on morphology and on physical and functional properties. Aquaculture Nutrition, 19(6): 845-859.

Draganovic, V.; Van der Goot, A.J.; Boom, R. and Jonkers, J. (2011). Assessment of the effects of fish meal, wheat gluten, soyprotein concentrate and feed moisture on extruder systemparameters and the technical quality of fish feed. Anim. Feed Sci.Technol., 165: 238–250.

Düsterhöft, E.M.; Voragen, A.G. and Engels, F.M. (1991). Non- starch polysaccharides from sunflower (Helianthus annuus) meal and palm kernel (Elaeis guineenis) meal—preparation of cell wall material and extraction of polysaccharide fractions. Journal of the Science of Food and Agriculture, 55(3): pp.411-422.

Gatrell, S.; Lum, K.; Kim, J. and Lei, X.G. (2014). Potential of defatted microalgae from the biofuel industry as an ingredient to replace corn and soybean meal in swine and poultry diets. Journal of Animal Science, 92(4): 1306-1314.

Glencross, B.; Hawkins, W.; Maas, R.; Karopoulos, M. and Hauler, R. (2010). Evaluation of the influence of different species and cultivars of lupin kernel meal on the extrusion process, pellet properties and viscosity parameters of salmonid feeds. Aquacult. Nutr. 16: 13–24.

Irish, G. G. and Balnave, D. (1993). Non-starch polysaccharides and broiler performance on diets containing soyabean meal as the sole protein concentrate. Australian Journal of Agricultural Research, 44(7): 1483-1499.

Jaworski, N. W.; Lærke, H.N.; Bach Knudsen, K.E.B. and Stein, H.H. (2015). Carbohydrate composition and in vitro digestibility of dry matter and nonstarch polysaccharides in corn, sorghum, and wheat and coproducts from these grains. Journal of animal science, 93(3): 1103-1113.

Kaisangsri, N.; Kowalski, R.J.; Kerdchoechuen, O.; Laohakunjit, N. and Ganjyal, G.M. (2019). Cellulose fiber enhances the physical characteristics of extruded biodegradable cassava starch foams. Industrial Crops and Products, 142: 111810.

Kallu, S.; Kowalski, R.J. and Ganjyal, G. M. (2017). Impacts of cellulose fiber particle size and starch type on expansion during extrusion processing. Journal of food science, 82(7): 1647-1656.

Knudsen, K. E. B. (2014). Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poultry Science, 93(9): 2380-2393.

Knudsen, K. E.B. (1997). Carbohydrate and lignin contents of plant materials used in animal feeding. Animal feed science and technology, 67(4): 319-338.

Kocher, A.; Choct, M.; Porter, M.D. and Broz, J. (2000). The effects of enzyme addition to broiler diets containing high concentrations of canola or sunflower meal. Poultry Science, 79(12): 1767-1774.

Korkerd, S.; Wanlapa, S.; Puttanlek, C.; Uttapap, D. and Rungsardthong, V. (2016). Expansion and functional properties of extruded snacks enriched with nutrition sources from food processing by-products. Journal of food science and technology, 53(1): 561-570.

Kraugerud, O.F.; Jørgensen, H.Y. and Svihus, B. (2011). Physical properties of extruded fish feed with inclusion of different plant (legumes, oilseeds, or cereals) meals. Animal Feed Science and Technology, 163(2-4): 244-254.

Martin, A.; Osen, R.; Greiling, A.; Karbstein, H. P. and Emin, A. (2019). Effect of rapeseed press cake and peel on the extruder response and physical pellet quality in extruded fish feed. Aquaculture, 512:734316.

Monti, M.; Gibson, M.; Loureiro, B. A.; Sá, F. C.; Putarov, T. C.; Villaverde, C. and Carciofi, A. C. (2016). Influence of dietary fiber on macrostructure and processing traits of extruded dog foods. Animal Feed Science and Technology, 220, 93-102.

Sanz, A.; Morales, A.E.; De la Higuera, M. and Gardenete, G. (1994). Sunflower meal compared with soybean meals as partial substitutes for fish meal in rainbow trout (Oncorhynchus mykiss) diets: protein and energy utilization. Aquaculture, 128(3-4), 287-300.

Senkoylu, N. and Dale, N. (1999). Sunflower meal in poultry diets: a review1. World's Poultry Science Journal, 55(2): 153-174.

Singh, S. K. and Muthukumarappan, K. (2016). Effect of feed moisture, extrusion temperature and screw speed on properties of soy white flakes based aquafeed: a response surface analysis. Journal of the Science of Food and Agriculture, 96(6): 2220-2229.

Sørensen, M. (2012). A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high- energy fish feed as measured by prevailing methods. Aquaculture nutrition, 18(3): 233-248.

Vehof, J.W.; de Ruijter, A.E.; Spauwen, P.H. and Jansen, J.A. (2001). Influence of rhBMP-2 on rat bone marrow stromal cells cultured on titanium fiber mesh. Tissue Engineering, 7(4): 373-383.