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Twin-screw Extrusion Processing of Vegetable-based Protein Feeds for Yellow Perch (*Perca flavescens*) Containing Distillers Dried Grains, Soy Protein Concentrate, and Fermented High Protein Soybean Meal

Parisa Fallahi[1], Kasiviswanathan Muthukumarappan[1], Kurt A. Rosentrater[2] & Michael L. Brown[1,3]

[1] Department of Agricultural and Biosystems Engineering, South Dakota State University, Brookings, SD, USA

[2] Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA, USA

[3] Department of Wildlife and Fisheries Sciences, South Dakota State University, Brookings, SD, USA

Correspondence: Kurt A. Rosentrater, Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA 50011, USA. Tel: 1-515-294-4019. E-mail: karosent@iastate.edu

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Abstract

Changing to alternative protein sources supports production of more economic aquafeeds. Two isocaloric (3.06 kcal/g) and isonitrogenous (40% db) experimental feeds for juvenile yellow perch were formulated with incorporation of fermented soybean meal (FSBM) and soy protein concentrate (SPC), each of which were at two levels (0 and 20% db), along with constant amounts of high protein distillers dried grains (DDG) (~30% db), and appropriate amounts of other ingredients. Using a pilot scale twin-screw extruder, feed production was performed in two replications for each diet at conditioner steam levels of 0.11 to 0.16 kg/min, extruder water of 0.11 to 0.19 kg/min, and screw speeds of 230 to 300 rpm. The effects of SPC and FSBM inclusion on extrudate physical properties were compared with those of a control diet (which contained 20% fishmeal and ~30% DDG). Inclusion of 20% FSBM and 20%SPC resulted in a substantial decrease in unit density by 9.2 and 24%, but an increase in lightness, greenness, yellowness, and expansion ratio of the extrudates by 7, 27, 14, 7, 17, 34, 15, and 16.5%, respectively. SPC inclusion led to a considerable increase in water absorption, thermal resistivity, and thermal diffusivity by 17.5, 6.3, and 17.6%, respectively, whereas no significant change was observed for these properties with incorporation of 20% FSBM. Additionally, all extruded products had high durability. Taken together, using ~30% DDG with20% FSBM or20% SPC as alternative protein sources resulted in viable extrudates with properties appropriate for yellow perch production. A future study investigating the effect of extrusion processing conditions on the production of complete vegetable-based protein feeds for yellow perch species would be appropriate.

Keywords: aquaculture, DDG, extrusion, fermented soybean meal, physical properties, soy protein concentrate

1. Introduction

Tremendous increases in global demand for production of seafood has resulted in the necessity of finding alternative, economic, and nutritious ingredients for aquafeeds. Nearly 60% of the average total costs of aquafarm production are accounted for by feed costs (Tan & Dominy, 1997), of which protein is the most costly ingredient. Traditionally, fishmeal is the main protein source required for aquafeeds and is obtained from wild fish, which can be one of the main pressing reasons for the high costs of the diets. Moreover, the importance of marine resource conservation and reducing the risks of environmental pollutions need to be considered.Currentlyconsidered. Currently, the goal is to minimize and even to eliminate fish meal inclusion in aqua diets by substituting appropriate alternative protein sources (Hardy & Masumoto 1990). Hence, production of appropriate aquafeeds depends on the type of fish species, which impacts both feed blend formulation and feed utilization.

Several research studies have been conducted to find alternative protein sources for fish meal, including protein obtained from plantsandplants and other livestock by-products, (Naylor et al., 2005). Most of these studies found that fish which were fed plant protein-based diets had less growth performance compared to those fed fish meal-based diets (Cho et al., 1974; Fowler & Banks, 1976). However, there are some studies that reported no adverse effects due to fish meal replacement by plant-based proteins (Garduño & Olvera, 2008). These contrasting results can be ascribed to better quality of plant-derived proteins; improved fish feed processing, and meeting all the nutritional requirements of the fish with appropriate supplementation of the diet (Adelizi et al., 1998).

Soy is one of the most abundant and protein-rich plantsinplants in the US, and it appears to be a promising source of protein for aquafeeds. Use of soy products such as full-fat soybean, toasted defatted soybean meal (SBM),untoasted defatted soybean meal or white flakes (WF), and mechanically oil extracted soybean cake in aquaculture feed production sis becoming common. In addition to protein, oil extracted from soybean is also a viable source of omega-3 fatty acids and fat that can be used instead of fish oil. Although soy products are potential sources of protein and oil for aquafeeds, compete with Fish meal, their inclusion is somewhat challenging due to lack of some essential amino acids (EAA) (like as lysine and methionine) and the presence of antinutritional factors (ANFs) such as trypsin inhibitors, phytates, glycinins, B-conglycinin, saponins, lectins,andlectins, and non-starch polysaccharides (NSPs) whichreducewhich reduce feed digestibilityanddigestibility and efficiency (Dabrowski et al., 1989; Olli & Krogdahl, 1994), and can cause gastrointestinal disturbances and intestinal damage. Therefore, attempts have been made to reduce the ANFs of conventional soybean products and to improve soy utilizations in both foods and feeds. For example, soy protein concentrate (SPC), soy protein isolate (SPI), and microbial fermented soy protein concentrate (FSPC) are modified forms of conventional soybean meal, in which the ANFs and NSPs have been reduced considerably (USSEC, 2012). Moreover, the concentrations of EAA in SPC and SPI are much higher than those of the original soy and even higher than those of the fish meal (FM). But high production cost of SPI and SPC can limit their utilization in aquafeeds at commercial scale.

SPC is the thermally-treated product of oil-extracted white soy flakes and it contains 65-67% crude protein. Moreover, due to thermal treatment, ANFs (e.g. trypsin inhibitors), antigens (e.g. glycinins), and NSPs (e.g. oligosaccharides) have been removed to a large extent (USSEC, 2012). Fermented soy protein concentrate is high protein SBM which is subjected to microbial or fungal fermentation, the ANFs are inactivatedclueinactivated due to enzymatic degradation (Hong et al., 2004), and maximum protein quality is retained due to low temperature exposure, and it contains approximately 54% crude protein. An added concern about inclusion of soy products in aquafeeds is the presence of lectin, a protein which is highly resistance to proteolytic enzymes. Exposure to high temperature (>100°C) for at least 5 min can destroy the lectin molecules; however, its activity is not influenced by some thermal processes like extrusion processing (Gatlin et al., 2007).

Most studies have been done to evaluate the effects of soybean products on growth performance of the aquafeed diets. Webster et al. (1992) could achieve an optimal growth rate for blue catfish, using a diet with 48% SBM along with 13% fish mealinclusionmeal inclusion. Kaushik et al. (1995) conducted research for partial and total replacement of fish meal with soy flours and SBM in rainbow trout diet, and reported that utilization of SBM and soy flour did not compromise the production performance of rainbow trout. Amaya et al. (2006) used combinations of SBM and corn gluten meal (CGM) as a complete fish meal replacer in shrimp diets and suggested that their proposed plantproteinplant protein-based diet could be used at commercial scale without any adverse effects.Ineffects. In another study, the effects of complete fishmeal replacement with SBM for Nile tilapia diets was investigated, and the optimal growth rate was observed for the diet supplemented with amino acids (El-Saidy et al., 2002). Similar results were obtained for the other fish species by Arndt et al. (1999) and Floreto et al. (2000). In general, successfulinclusionsuccessful inclusion of plant-based proteins (such as soy and corn products) in aquafeeds can be achieved with the use of amino acid supplements (Adelizi, et al., 1998; Ayadi et al., 2011a, b, c).One of the drawbacks of soy-based protein products is their relatively high trypsin inhibitor activities. Protease inhibitor activity of SBM-based fish feed and the related negative effects on growth rate and protein efficiency ratio of fingerling channel catfish were extensively studied by Wilson & Poe (1985) and Perse et al. (2003).The nutritional qualities of the diets including WF and SBM as FM replacers were also assessed in experiments with rainbow trout (Romarheim et al., 2006) and cobia (Romarheim et al., 2008) species.

Yellow perch (*Perca flavescens*) are a major speciesofspecies of the Great Lakes region, and are a traditional consumer favorite. Decreasing population of this small-sized species and increased consumer demands have increased its potential for aquaculture, particularly in the north central region of the US (Gonzalez, et al., 2006).While there has been much research into nutritional requirements and utilization of alternative protein sources for species like Nile tilapia, channel catfish, shrimp, salmon and other commonly eaten fish, studies for yellow perch are very limited. Nutritional requirements of yellow perch were determined by Reinitz and Austine (1980). Required protein content for optimum growth rate of yellow perch with size of 18.6-27.1 g was reported in the range of 21-27% (Ramseyer & Garling, 1998). Artola (2004) investigated the effects of defatted-soybean meal inclusion with and without supplemental lysine in yellow perch diets, and examined weight gain, feed efficiency ratio, water quality of the pond, and final quality of the fish fillets. They concluded that the inclusion of soybean meal supplemented by lysine had no significant impact on the product characteristics; but total nitrogen content of the waste effluent was decreased substantially using soy.Insoy. In another study, growth performance of yellow perch fed varyinglevelsvarying levels of two types of SBM (solvent-extracted, dehulled soybean meal (sed-SBM) and expelled-extruded soybean meal (ex-SBM)) were studied (Kasper et al., 2007). They observed that growth rate was significantly influenced by the type of SBM, concentration used and their interactions. Overall they achieved optimal growth rate at 30% SBM inclusion for both types of SBMs. Schaeffer et al. (2011) performed a feeding trial study to assess the effects of diets containing DDGS (distillers dried grains with solubles) in combination with SBM on final pellet quality, growth rate parameters,andparameters, and digestibility for juvenile yellow perch,andperch, and determined up to 49.5% combined inclusion of DDGS and SBM could be suitable for yellow perch diets.

In fact, a well-balancednutritionalbalanced nutritional feed is just one of the crucial factors contributing to optimal fish growth performance. The other important factor is the method of feed manufacturing. Extrusion cooking is the most common and effective method used in the aquafeed industry. An extruder is a high temperature, short time bioreactor that performs work on the feed ingredients using thermal and mechanical energy, and converts the ingredients into dough and shapes the melted dough through a die restriction. Its performance is influenced by several dependent and independent variables such as feed composition, moisture content, barrel temperature, screw speed, screw configuration, die size, mass flow rate, and residence time in the extruder.Theextruder. The interaction of high shear and temperature developing inside the extruder with water drastically affects the molecular structure of the material, mainly due to gelatinization of starch and denaturation of protein components of the feed blend,whichblend, which influences the resulting physiochemical and nutritional properties of the extrudates (Guy, 2001). Optimal process conditions (Banerjee & Chakraborty, 1998; Rolfe et al., 2000) coupled with an appropriate feed blend composition (Cavalcanti & Behnke, 2005a, b) need to be applied to achieve physically and nutritionally viable aquafeeds. Desired physical properties of the extruded aquafeed, like the nutritional requirements of the fish, vary depending upon the fish species. Typically, high water stability, maximum unit density and expansion ratio play the crucial roles in extruded aqua diets (Wood, 1995), since the high water stability prevents rapid disintegration and loss of nutrients of the feed when placed in water, and thus the feed is available for the fish for a longer period. On the other hand, expanded products have experienced more starch gelatinization during the process and may be more digestible to fish (Chang & Wang, 1999; Gokulakrishnana & Bandyopadhyay, 1995).

The effect of extrusion cooking on DDGS and soy-based diets for Nile tilapia and channel catfish were extensively studied by Chevanan et al. (2007a; 2007b; 2007c; 2008; 2009; 2010), Kannadhason et al. (2009; 2010; 2011), Fallahi et al. (2011), and Rosentrater et al. (2009a; 2009b; 2010). In a feeding trial conducted by Schaeffer et al. (2011) the effect of SBM and DDGS combinations on the physical properties of yellow perch diets extruded in a single screw extruder was investigated. Ayadi et al. (2011a; 2011b; 2011c) examined the effect of DDGS inclusion on the physical properties of the extruded yellow perch and Rainbow trout diets. Mjoun and Rosentrater (2011) thenconductedthen conducted a twin screw extrusion experiment to produce yellow perch diets including up to 50% DDGS, and studied the effect of DDGS on physical properties of the product and process parameters.

The effect of partial replacement of FM with either SBM or WF on physical properties and extrusion parameters of Salmonid dietswerediets were evaluated by Sørensen et al. (2009) .They concluded that partial inclusion of WF or SBM could improve the physical properties of the extrudates in general (i.e. higher durability, higher breaking force toleration, and higher bulk density); however, they observed a considerable decrease in expansion ratio of the extrudates.

Effect of dietary supplementation of fermented soybean meal (FSBM) on juvenile parrot fish (*Oplegnathus fasciatus*) growth performance was studied by Kim et al. (2009). They suggested that inclusion of fermented soybean meal could improve the phosphorous absorption by fish.

So far, only a few researchstudiesresearch studies have explored the effect of soy protein inclusion on physical properties of aquafeeds produced by the extrusion cooking process. Moreover, no study has been conducted on the effects of fermented soy products (such as FSBM) inclusion on physical properties of aquafeeds.Toaquafeeds. To our knowledge, the research for physical properties of the soy protein-based yellow perch diet is even more scarce. Thus, the goals of this study were: 1) to produce a complete vegetable -based feed for Juvenile yellow perch species with proportional SBM and FSBM inclusions; 2) to investigate the effect of FSBM and SPC on the physical properties of the extruded feeds.

2. Materials and Methods

2.1 Experimental Design and Sample Preparation

Two isocaloric (3.06 kcal/g) experimental diets containing approximately constant high protein distillers dried grains (HP-DDG) (~30% db), two levels of microbial fermented high protein soybean meal (FSBM) and soy protein concentrate (SPC) (0 and 40% db), in combination with appropriate amounts of the other required ingredients including corn gluten meal, wheat flour, vitamin mix, minerals, and essential amino acids, were formulated to contain a target protein content of ~40% db (Table 1).

Table 1. Ingredient components (g/100g) and nutrient compositions (% db) of the feed blends

Components (%db)	**Control**	**Diet 1**	**Diet 2**
Dry weight of ingredients (g/100g)			
HP DDG[a]	30.99	31.46	31.45
PepSoyGen[b]	0.00	21.16	0.00
Solae SPC[c]	0.00	0.00	21.17
Fish meal[d]	20.39	0.00	0.00
Corn gluten meal[e]	14.97	16.71	16.71
Whole wheat flour[f]	22.96	20.77	20.77
CMC[g]	6.71	1.70	1.70
Vitamin premix[h]	0.56	0.57	0.57
Mineral mix[i]	0.11	0.11	0.11
Oils	—	—	—
Supplements (total from below)	3.36	8.67	7.51
Stay-	0.06	0.06	0.06
Choline	0.23	0.23	0.23
Phytase	0.04	0.04	0.04
DVAqua	0.14	0.14	0.14
Arginine	0.00	0.57	0.28
Lysine	0.00	1.70	0.85
Isoleucine	0.00	0.06	0.00
Histidine	0.00	0.11	0.00
Glycine	0.57	0.57	0.57
Methionine	0.00	0.51	0.28
Taurine	0.00	0.00	0.00
Sodium chloride	1.13	1.13	1.14
Potassium chloride	0.91	0.91	0.91
Magnesium oxide	0.06	0.06	0.06
Calcium phosphate	0.23	2.60	2.95
Total	100.00	100.00	100.00
Feed blend composition (% db)			
Crude Protein	37.18	40.6	43.27
Crude Fat	7.52	7.83	7.82
Crude Fiber	0.24	0.24	0.24
Ash	2.73	4.53	4.53

[a] POST (Sioux Falls, SD)

[b] PepSoyGen, fermented high protein soybean meal, NutraFerma (Sioux City, IA)

[c] Solae, soy protein concentrate (SPC), Solae, LLC(St. Louis)

[d] Menhaden fish meal, Omega Protein Inc. (Houston, TX)

[e] Consumers Supply Distributing Company (Sioux City, IA)

[f] Bob's Red Mill Natural Foods, Inc. (Milwaukie, OR)

[g] Carboxyl methyl cellulose (CMC), USB Corporation (Cleveland, OH)

[h] Lorscher Agri Service, Inc. (Bern, KS)

[i] Lorscher Agri Service, Inc. (Bern, KS)

HP-DDG was provided by Poet (Sioux Falls, SD), and was ground to a fine particle size of approximately 100 µm with a laboratory-scale grinder (Model S500 disc mill, Gen Mills, Clifton, NJ). PepSoyGen was purchased from NutraFerma (Sioux City, IA); SPC from Solae, LLC (St. Louis, MO); corn gluten meal from Consumers Supply Distributing Company (Sioux City, IA); wheat flour from Bob's Red Mill Natural Foods, Inc. (Milwaukie, OR); CMC from USB Corporation (Cleveland, OH); vitamin premix and minerals were from Lortscher Agri Service, Inc. (Bern, KS). The ingredients were mixed with a laboratory-scale mixer (Model 600, Hobart Corporation, Troy, OH) for 3 min; then, the vitamin premix was added to the rest of the ingredients, and the blend was mixed with a twin shell dry blender (The Patterson-Kelly Co. Inc., East Stroudsburg, PA) at 60 rpm for 10 min to produce homogenous blends. The resulting blendswereblends were then stored at ambient temperature overnight.

2.2 Extrusion Processing

Extrusions were carried out using a pilot scale, self-wiping, fully intermeshing, co-rotating twin-screw extruder (Wenger TX-52, Sabetha, KS) with a 30 hp motor and throughput of 50 - 250 kg / h. The extruder was equipped with a dry feed hopper and a continuous preconditioner. Using the feed hopper, the dry ingredient blends were transferred and conveyed into the preconditioner where the desired moisture content and temperature were adjusted via steam injection at a rate of 0.11-0.16 kg/min. The blends were transferred into the extruder at a feeder rate of 20 kg/h. The screws' diameter and L/D ratio of the extruder barrel were 52 mm and 25.5:1, respectively. The screws used in this experiment had 25 individual sections, and the configuration from the feeding to the die section, was composed of: four conveying screws, three shear locks, one conveying screw, one conveying screw backward, three conveying screws, one conveying screw backwards, four conveying screws, one shear lock, one interrupted flight conveying screw, one conveying screw, one interrupted flight conveying screw, one conveying screw, one interrupted flight conveying screw, one shear lock, and finally a screw with a cone-shaped end point. Moreover, the barrel was composed of eight temperature zones which were set at 25 to 90 ^{0}C. The temperature profile of the barrel varied, depending on the actual temperature of each zone and the extrudates' properties. The amount of water added to the extruder was maintained at0.11to0.19 kg/min. It had two die nozzles each of which with circular opening of 3 mm. The exiting extrudates were cut into desired lengths, using a three blade cutter at the end of the die.

2.3Measurement of Extrusion Processing Parameters

2.3.1 Temperature (T)

Temperatures of the raw blends inside the hopper, at the conditioner exit and die zone were all monitored by a portable infrared thermometer (Model 42540, Extech Instruments Corporation, Waltham, MA).

2.4 Measurement of Extrudate Physical Properties

The extruded diets were cooled for 72 h at ambient temperature (24±1^{0}C) and dried in an oven (Model TAH-500, Grieve Corporation, Round Lake, IL) for 24 h at 45^{0}C. Then, the dried extrudates were subjected to extensive physical property analyses, including moisture content (MC), water activity (a_w), thermal conductivity (K), thermal resistivity (R), thermal diffusivity (α), expansion ratio (ER), unit density (UD), bulk density (BD), water absorption and water solubility indices (WAI, WSI), pellet durability index (PDI), and color,followingcolor, following the methods delineated by Rosentrater et al. (2005).

2.4.1 Moisture Content (MC)

MC of the extrudates was determined according to AACC method 44-19 (2000), using a laboratory-scale oven (Fischer Scientific) at 135 ^{0}C for 2h.

2.4.2 Water Activity (a_w)

Water activity of the extrudates was measured with a water activity meter (a_w Sprint TH-500, Novasina, Pfäffikon, Switzerland). The system was calibrated according to the procedure specified by the manufacturer.

2.4.3 Thermal Properties

Thermal conductivity (k), thermal diffusivity (α), and thermal resistivity (R) were determined using a thermal properties analyzer (KD2, Decagon Devices, Inc., Pullman, WA).

2.4.4 Expansion Ratio (ER)

The diametral expansion of the extrudates was determined as the ratio of extrudate diameter (mm) to the diameter (mm) of the die nozzle, using a digital caliper (Digimatic Series No. 293, Mitutoyo Co., Tokyo, Japan.).

2.4.5 Unit Density (UD)

Assuming cylindrical shapes for the extrudates, UD was determined as the ratio of the mass (g) to volume (cm^3) for ten randomly chosen extrudates. The mass of each of the extrudates was measured with an analytical balance (Adventurer. AR 1140, Ohaus Corp. Pine Brook, NJ) and the diameter of each was measured with a digital caliper (Digimatic Series No. 293, Mitutoyo Co., Tokyo, Japan).

2.4.6 Bulk Density (BD)

Using a standard bushel tester (Seedburo Equipment Company, Chicago, IL), BD (g/cm^3) was measured as the ratio of the mass of the extrudates (g) occupying a given bulk volume, to the volume of the bulk (0.5 L) (USDA, 1999).

2.4.7 Water Absorption and Water Solubility Indices (WAI, WSI)

Using a laboratory–scale grinder (Chemical Rubber Co, CRC, Germany) extrudate samples were ground to a particle size of approximately 150 µm,thenm, then 2.5 g of the finely ground sample were placed in a 50 mL centrifuge tube, and 30 mL distilled water at 30 ^{0}C was added to the tube. After intermittently stirring for 30 min, the suspension was centrifuged at 3000 X g for 15 min using a laboratory-scale centrifuge (accuSpinTM 400, Thermo Electron Corporation). Thereafter, the supernatant phase was transferred into aluminum dishes and placed in a laboratory oven (Fisher Scientific) at 135^{o}C for 2h. WAI was calculated as the mass ratio of the remaining gel in the centrifuge tube to the original mass of the sample, equation (1):

$$WAI(-) = (Mass\ of\ gel) / (Mass\ of\ sample) \tag{1}$$

Where masses were determined in g.

Subsequently, WSI (%) was calculated as the mass ratio of the extracted dry solid to the original sample mass, following Anderson et al. (1969).

2.4.8 Pellet Durability Index (PDI)

PDI was determined following ASAE standard method S269.4 (ASAE, 1994); 200 g of the extruded sample was tumbled inside a PDI tester (model PDT -110, Seedburo Equipment Co., Chicago, IL) for 10 min, and then sieved manually via a No. 6 screen. PDI was calculated with equation (2), where M_a and M_b are the mass (g) of the extrudates after tumbling and before tumbling, respectively:

$$PDI(\%) = (Ma / Mb) \times 100 \tag{2}$$

2.4.9 Color

Color included L* (brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness). These parameters were measured using a spectrophotometer (Lab Scan XE, Hunter Lab, Reston, VA).

3. Statistical Analysis

All measurements were made in triplicate, except for UD and ER, where ten measurements were taken. All collected data were analyzed with Microsoft Excel v.2010 and SAS v.9.0 software (SAS Institute, Cary, NC) using a Type I error rate (α) of 0.05, by analysis of variance (ANOVA) to find if there were significant differences among control diet and experimental diets. Then post-hoc Duncan tests were used to determine where the specific differences occurred.

4. Results and Discussion

4.1 Extrusion Processing Parameters

4.1.1Temperature during processing (T)

Temperatures at three points of the process were monitored. As shown in Table 2, inclusion of 20% FSBM led to a 10% increase in processing temperature at conditioner section (T_C) and had no impact on die temperature (T_d), where addition of 20% SPC resulted in a 19.5% increase in T_C but an 8% drop in die temperature (T_d). Overall, substitution of SPC significantly decreased the T_d of the extrudates while no considerable change was observed for that of produced by 20% FSBM. Proportional increasing rate of temperature along the extruder length can be ascribed to both heat addition as well as frictional heating.

Table 2. Treatment effects on extrusion processing parameters

	Treatment		
Parameter	Control	Diet1	Diet 2
Processing temperature (°C)			
Feeder zone	21.84^{b}	22.53^{b}	23.55^{a}
	(0.51)	(0.60)	(0.85)
Conditioner zone	22.66^{b}	24.95^{a}	27.07^{a}
	(0.17)	(2.42)	(2.11)
Die zone	47.32^{a}	47.74^{a}	43.51^{b}
	(4.80)	(3.13)	(2.57)

Mean values among treatments followed by similar letters for a given dependent variable are not significantly different at $P<0.05$. Values in parentheses are standard deviation.

4.2 Extrudate Physical Properties

All the measured physical properties of the final products including moisture content, water activity, thermal properties, expansion ratio, unit density, bulk density, water absorption and water solubility indices, pellet durability index, and color are provided in Table 3.

4.2.1 Moisture content (MC)

Moisture content within a substance can exist in three forms of bound, unbound, and free water (Mohsenin, 1986). Free moisture content is the amount of moisture higher than the equilibrium moisture content and can be removed by drying under a definite relative humidity. In extrusion cooking process like the other food and feed processes, moisture content of the raw materials and final products plays a crucial role, as it affects the finished products physical properties like cohesiveness, water stability, and storage stability due to its impacts on extrusion processing behavior (Rolfe et al., 2001).

Typically, biochemical reactions among the water, starch, and protein contents of the raw blend are profoundly influenced by the interaction effects of thermomechanial phenomena like heat transfer, shearing forces, and high pressure developed inside the barrel of the extruder during the process. As a result of these complex biochemical and thermomechanial interactions; the pressure difference among the die entrance, die exit, and atmosphere, the internal structural of the molten dough changes and free water content of the melt evaporates and flashes off into the vapor phase. This water alteration affects the extrudates' properties. However, the amount and nature of the starch and protein contents of the blend arealsoare also important (Miller 1985; Riaz, 2000; Mercier et al., 1989), since, the extent of starch gelatinization and protein denaturation are the results of interactions among the pressure difference, heating, shearing and water content during evaporation (Friesen et al., 1992).

As depicted in Table 3, inclusion of FSBM and SPC resulted in 23.8% and 13.8 % increase in MC of the extrudates compared to that of the control diet, respectively. No significant influence on MC of the products was observed between the two experimental diets.

4.2.2 Water Activity (a_w)

Using SPC and/or FSBM in the diet formula doubled the a_w of the extrudates compared to that of the control diet. But no significant difference between the a_w of diet 1 and 2 extrudates was detected (Table 3).

As per the quantitative definition, a_w refers to the ratio of water vapor pressure in a material to that of pure water at the same temperature and pressure (Koop et al., 2000). The amount of water available for microorganism use is a crucial consideration for food and biological material safety. Due to variations of water binding with temperature, water activity of materials is a temperature-dependent factor (Higl et al., 2007); therefore, it can impactenzymeimpact enzyme activity, vitamin availability, physical state of bacterial survival, and consequently the shelf life of biological materials. The lower the water activity of material, the less risk of spoilage.

Table 3. Treatment effects on extrudate physical properties

Property	**Treatment**		
	Control	Diet 1	Diet 2
MC (%db)	4.41^{b}	5.46^{a}	5.02^{ab}
	(0.85)	(0.45)	(0.23)
a_w (-)	0.17^{b}	0.25^{a}	0.25^{a}
	(0.01)	(0.01)	(0.01)
k (W/m·°C)	0.05^{a}	0.05^{a}	0.05^{a}
	(0.01)	(0.01)	(0.01)
R (m·°C/W)	19.87^{b}	19.87^{b}	21.13^{a}
	(0.72)	(0.95)	(0.94)
α (mm^2/s)	0.17^{b}	0.18^{b}	0.2^{a}
	(0.01)	(0.01)	(0.01)
ER (-)	1.15^{c}	1.23^{b}	1.34^{a}
	(0.07)	(0.08)	(0.09)
UD (kg/m^3)	662.46^{a}	601.43^{b}	504.02^{c}
	(78.17)	(67.02)	(68.87)
BD (kg/m^3)	514.88^{a}	478.78^{a}	417.5^{b}
	(1.07)	(5.12)	(60.31)
WAI (-)	3.09^{b}	2.93^{b}	3.63^{a}
	(0.03)	(0.13)	(0.66)
WSI (%)	8.29^{b}	10.04^{a}	8.72^{b}
	(0.55)	(0.79)	(0.78)
PDI (%)	99.53^{a}	99.23^{ab}	99.13^{b}
	(0.08)	(0.02)	(0.43)
L* (-)	26.84^{c}	28.7^{b}	31.31^{a}
	(0.20)	(0.53)	(2.20)
a* (-)	6.84^{c}	8.68^{b}	9.19^{a}
	(0.03)	(0.10)	(0.10)
b* (-)	11.84^{c}	13.5^{b}	15.54^{a}
	(0.09)	(0.15)	(1.30)

MC is moisture content; a_w is water activity; K k is thermal conductivity; R is thermal resistivity; α is thermal diffusivity; ER is expansion ratio; UD is unit density; BD is bulk density; WAI is water absorption index; WSI is water solubility index; PDI is pellet durability index; L* is brightness /darkness; a* is redness/greenness; b* is yellowness/blueness; Mean values among treatments followed by similar letters for a given dependent variable are not significantly different at P<0.05. Values in parentheses are standard deviation.

4.2.3 Thermal Properties

Thermal properties of the extruded plant-protein based diets included thermal conductivity (k), thermal diffusivity (α), and thermal resistivity (R) were measured and the data are provided in Table 3. Neither inclusion of SPC nor FSBM changed the k value of the extrudates compared to that of the control diet. Thermal conductivity is a temperature-dependent property, which indicates a material's potential for transferring heat thorough itself by conduction (i.e. without any motion). Heldman (2003) suggested that the k value of aheata heat processed material decreasedduedecreased due to protein denaturation.

Addition of SPC increased the thermal resistivity and diffusivity of the extrudates by 6.3% and 17.6%, respectively. But using FSBM did not result in any significant impact on the thermal properties of the extrudates. Thermal resistivity indicates the ability of a material to prevent the heat transfer through that material, and it is dependent on the material thickness and the temperature gradient across the material (Arambula-Villa et al., (2007). Kawasaki and Kawai (2006) suggested that thermal diffusivity is related to the capability to store the heat. The low k value and α value of the raw blends may affect the cooking process adversely (Ayadi et al., 2011a), since, the blends used requires a longer time to transfer heat. They also suggested that extrusion cooking process of the blends with low heat conductivity and diffusivity may be more stable due to being less affected by external thermal sources. Additionally, the low values of thermal properties can influence the required time for cooling and/or drying of the extrudates.

On the other hand, there was a positive relation between the ER and α values of the plant-protein based extrudates compared to that of the control diet (Figure 1). The higher α value, the more the ER. These observations can be ascribed to the higher porosity of the more expanded extrudates. SincetheSince the thermal diffusivity of the air is larger than those of the protein and starch by more than double order of magnitude (Mariam et al., 2008). Addition of more hydrophobic ingredients in the diet can decrease the k value of the extrudates. This explains why thermal properties of the extrudates are highly related to the diet formulation, extruder operational conditions, and internal structure of the extrudates (Alavi et al., 1999). Knowledge of the thermal properties of the ingredients can be helpful to control the extrusion operational conditions and to manipulate the product physical properties such as expansion ratio and density.

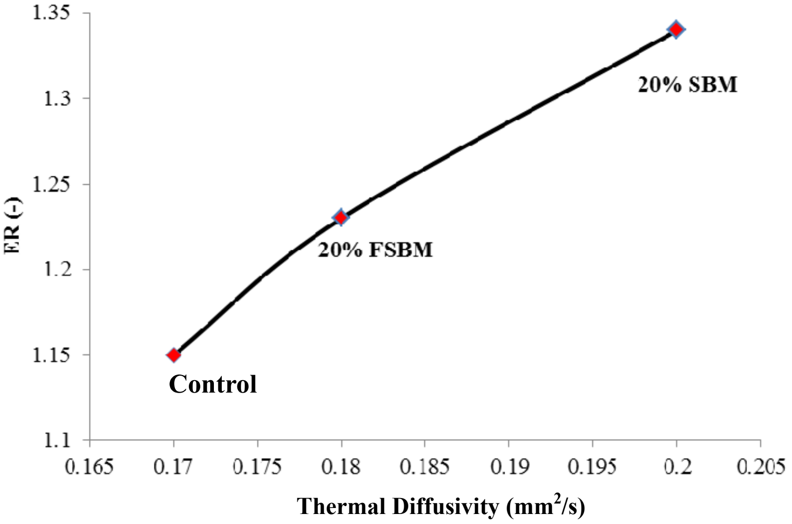

Figure 1. Relation between extrudate thermal diffusivity and expansion ratio (ER)

4.2.4 Expansion Ratio (ER)

Among the aquafeed physical characteristics,expansioncharacteristics, expansion ratio is one of the most important properties which has wide-ranging impacts the quality of the feed. ER influencesaninfluences an extrudate'sextrudate' s floatabilityandfloatability and brittleness (Rosentrater et al., 2009a; 2009b; Rolfe et al., 2001). Moreover, higher ER can improve the feed digestibility.ER is mainly impacted by the extent of pressure developed at the die exit during the evaporation phenomenon, which by itself is influenced by several factors such as the dough moisture content, die dimensions, and feed ingredient composition and mass flow rate (Tumuluru & Sokhansanj, 2008; Chevanan et al., 2007a), residence time, and rheological behavior of the dough (Fan et al., 1994; Mitchell et al., 1994; Chevanan et al., 2007a), respectively.Therespectively. The interaction effects of these variables at high levelsoflevels of temperature and shear forces during extrusion processing change the internal structure of the dough during the cooking process inside the barrel and during the evaporation process at the die exit. Expansion is mainly governed by the sudden decrease in pressure attheat the die section, which results in water phase transition and formation of air cells in the extrudates (Alves et al., 1999; Lam & Flores 2003; Moore, 1990; Chevanan et al., 2007a).Additionally, during the cooking process, the melted dough exhibits a pseudoplastic behavior and thus, a substantial decrease in the apparent viscosity of the dough, which is influenced by the temperature, moisture content and type of the ingredients and ultimately affects the extent of pressure release and expansion of the finished product (Rosentrater et al., 2005; Chevanan et al., 2007a). In general, the more moisture, the less expansion due to the lubricant effect of water and consequent decrease in apparent viscosity of the dough (Mjoun & Rosentrater, 2011); the higher the starch content, the greater the expansion due to starch gelatinization and elastic behaviour of the dough inside the barrel and consequent pressure rise at the die exit (Nielsen, 1976; Kokini et al., 1992; Case et al., 1992; Sokhey et al., 1994; Ibanoglu et al., 1996). Kim et al (1989) suggested that increasing the extent of expansion as a result of increase in barrel temperature could be related to more starch gelatinization and more excretion of super-heated steam. On the other hand, the more protein inclusion in the ingredient mix results in production of more porous extrudates due to the protein denaturation and plastic behaviour of the melted dough inside the extrusion barrel (Singh et al., 1991; Sandra & Jose, 1993; Chevanan et al., 2007a).

As depicted in Table 3, inclusion of 20% FSBM and 20% SPC resulted in a significant increase in extrudate ER by nearly 7% and 16.5%, compared to that of the control diet, respectively. Also, it is clear that the average ER of the extrudates from diet 2 was 9% higher than that of the diet 1, indicating that inclusion of SPC led to the production of more buoyant extrudates which might float for a longer period on the surface of the pond or aquarium. The higher ER of the extrudates from Diet 2 is maybe due to the lower crude fiber content of SPC compared to that of FSBM which would reduce moisture desorption.

4.2.5 Unit Density (UD)

Comparison of the UD of the extrudates from the two experimental diets to that of the control diet,itdiet, it appearsthatappears that the inclusion of SPC and FSBM resulted in decreased UD by 24% and 9.2%, respectively (Table 3). Overall, UD values were less than that of the water which confirms the buoyancy of the extrudates and was in agreement with the results obtained for ER values (i.e., there was an inverse correlation between the UD and ER values of the all extrudates), as was expected (Bhatnagar & Hanna, 1986; Colonna et al., 1989).

SPC had a greater effect than FSBM, however, Similar to ER, unit density of the extrudates is influenced by the physiochemical and thermomechanial alterations occurring inside the barrel of the extruder as a result of interactions among various dependent and independent variables. For example, Tumuluru and Sokhansanj (2008) observed that screw speed, barrel temperature, die dimension, and feed moisture content significantly influenced the UD values of the extrudates. They proposed that feed blend moisture content played a substantial role in cohesiveness of the extrudates, and affected the UD of the product due to its significant impact on starch gelatinization and providing the binding conditions for the gelatinized starch, the proteins, and other ingredients of the diet. In other words water acted as a plasticizer. Inplasticizer. In contrast, Chevanan et al. (2007a) observed that increasing the MC had no considerable effect on UD, whereas the barrel temperatureappearedtemperature appeared to have inverse effect on UD of the extrudates. Their results were in agreement with what Bhattacharya and Hanna (1986) reported. They believed the effect of temperature on the UD values of the extrudates could be ascribed to the decrease in apparent viscosity of the dough and lower expansion ratio occurring at the die exit. In other studies conducted by Rosentrater and Tulbek (2010) and Fallahi et al. (2011), the effect of some of the extrusion processing parameters, includingconditionerincluding conditioner steam, conditioner water, and screw speed, on the properties of extruded DDGS- based aquafeeds were investigated. Fallahi et al. (2011) observed that increasing screw speed led to a 10% decrease in UD of the extruded Nile Tilapia diets.

4.2.6 Bulk Density (BD)

From a commercial point of view, BD is a key propertyofproperty of biological products, since it dictatesthedictates the requiredstoragerequired storage space for a given product mass (Guy, 2001; Rosentrater 2006). Hence, the higher the bulk density, the lower the packaging, storage and transportation costs for a given mass of product.

As shown in Table 3, the lowest BD of 417.5 kg/m^3 was observed for the extrudates with 20% SPC inclusion (Diet2), while the highest BD value of 514.88 kg/m^3 was obtained for the control diet. There was no significant difference between the BD values of extrudates with 20% FSBM and that of the control diet. These observations for the increasing and decreasing values of ER and UD of the extrudates with 20% SPC inclusion confirm the direct and inverse relation of BD to UD and ER, respectively. Since the most floatable extrudates had the lowest BD and UD values, it is crucial to make a balance between the desired ER and BD values of the products (Mjoun & Rosentrater, 2011).

4.2.7 Water Absorption and Water Solubility Indices (WAI, WSI)

Table 3 shows that inclusion of 20% SPC resulted in 17.5% increase in WAI compared to that of the control diet, while inclusion of 20% FSBM did not show a significant effect on WAI of the extrudates. The maximum and minimum values of the WAI were 3.63 and 2.93, respectively. Additionally, there were inverse and direct relationships between the WAI and BD, and between the WAI and ER of the extrudates, respectively, implying that extrudates with the lowest BD and the highest ER appeared to have the highest WAI. Diet 2 with the lowest BD (417.5 kg/m^3) has maximum ER and WAI of 1.34 and 3.63, respectively. Thus, the more extrudates expanded, the more water was absorbed by the extrudates. Our observations in this regard are in agreement with what Adeparusi and Famurewa (2011) reported. Typically, WAI value indicates the portion of starch content of the feed blend which was not damaged by the extrusion cooking process and thus retained their internal structure (Govindasamy, 1996; Chevanan et al., 2007a). Several researchers suggested that any change in WAI can be due to structural modifications of the blend composition, such as starch gelatinization and protein denaturation (Badrie & Mellowes, 1991; Chevanan et al., 2007a; Rosentrater et al., 2009a; 2009b). Unlike water absorption index, WSI indicates degradation extent of macromolecule components of a feed blend, mainly starch and protein molecules (Govindasamy, 1996; Colonna Mercier, 1983). Therefore, WAI is inversely related to WSI (Anderson et al., 1969). Generally, extrusion processing increases WSI (Anderson et al., 1969), which occurs due to the combination effects of high temperature, pressure, and shear forces on starch and protein degradations. In this study, maximum WSI value of 10% was related to the diet with 20% FSBM inclusion, which increased by 21% compared to that of the control diet. The 20% SPC inclusion did not show considerable impact on WSI compared to WSI of the control diet.

4.2.8 Pellet Durability Index (PDI)

Durability of the extrudates during storage, transport, and feeding can be evaluated by PDI measurement. Basically, it indicates the mechanical strength of each extruded product against external forces, which is influenced by the intensity of heat treatment, along with the level of starch transformation due to the simultaneous impacts of barrel temperature and feed blend moisture content (Colonna et al., 1989; Chevanan et al., 2007; Rosentrater et al., 2009a; 2009b). As shown in Table 3, Diet 2 with 20% SPC inclusion exhibited the lowest PDI; however, there was no statistical differences between the PDI values of the experimental diets compared to that of the control diet. All the extrudates were highly durable and had PDI values of more than 99%.

4.2.9 Color (L*, b*, a*)

The effects of each diet on color of the extrudates are provided in Table 3. Inclusion of soy protein products in yellow perch diets significantly increased L*, a*, and b* values of the extrudates by 7, 27, and 14% for FSBM–based diets; by 17, 34, and 15% for the SPC-based diets, respectively. Generally, color of feed and biological products is an important quality (Ilo & Berghofer, 1999), which also can be indirectly related to the nutritional value of the products (Rosentrater et al., 2005; Berset 1989). Color of the feeds is greatly affected by the operating conditions of the applied process. In extrusion cooking, the degree of chemical and structural alterations of the dough inside the extruder barrel are the decisive factors in quality of the extrudates,whichextrudates, which by themselves are influenced by the degree of heat treatment, intensity of the shear forces, and composition of the ingredients. With regard to color quality, the most common reactions occurring during the residence of the dough inside the barrel that can alter the color are Maillard reactions and protein denaturation (Rosentrater et al., 2009a; Bjorck et al., 1985; Ilo & Berghofer, 1999). Goedeken (1991) suggested that protein denaturation and nonenzymatic browning reactions (Maillard) are influenced by combination effects of high temperatures and low moisture. However, the effect of ingredient composition of the mixtures and their natural colors need to be considered. Maillard reaction can destroy the amino acid chains of the protein molecules (Rosentrater et al., 2005) and thus decrease the protein digestibility. Finot (1982) suggested thatformationthat formation of an amadori complex of lysine amino acid at the early stage of the Maillard reaction can block this basic amino acid and makes it biologically unavailable,whichunavailable, which reduces the nutritional quality of the food. Chevanan et al. (2007c) suggested that changes in color of extruded products may be an indication of lysine alterations.

4.2.10 Other considerations

Indeed, there were many differences among the pelleted feeds in terms of physical properties. Ultimately, however, consumption by the fish and their resulting growth performance are critical to the success of any feed development effort. Although not reported here, we have simultaneously conducted a feeding trial using these feeds, in order to determine the nutritional effectiveness of these feeds. That information will be published elsewhere.

5. Conclusion

Using SPC and FSBM as fishmeal replacers in yellow perch diets led to a decrease in unit and bulk densities, but an increase in expansion ratio. Replacing fishmeal with 20% SPC and 20% FSBM resulted in increased water absorption and increased water solubility, respectively. Both plant protein–based diets exhibited increased color values of the extrudates including darkness (L), redness (a), and yellowness (b). Extrudates with SPC inclusion appeared to have the higher darkness value. High values of PDI for the extrudates of both experimental diets indicate that SPC and FSBM inclusions do not have detrimental influences on the mechanical strength of the yellow perch extruded diets. To sum up, high-quality extrudates were achieved in this studyandstudy and can be used in the aquaculture industry. Further extrusion studies are needed to investigate the effects of extrusion processing conditions on the production of complete vegetable-based protein feeds for yellow perch species, and to determine the optimal diet, which still require feeding trials.

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