Twin-screw Extrusion Processing of Vegetable-Based Rainbow trout (Oncorhynchus mykiss) Feeds Using Graded Levels of High Protein Fermented Soybean Meal (FSBM)

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Keywords
Aquaculture, Extrusion, PSG, Rainbow trout, Twin-screw Extruder

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Introduction

In response to the burgeoning rate of world population growth, researchers continue to seek to provide an adequate and secure supply of food. Seafoods provide many protein sources that can be used to help these needs. However, the sustainability of this valuable food source and the impact of edible marine fish overharvesting on ecosystems have become valid concerns. In 2009, the United States ranked third worldwide in consumption of fish and seafood, yet 80% of these products were imported, which makes it second only to oil imports (NOAA, 2010). In 2011, the total value of fishery product imports (i.e. edible and non-edible) was $30.8 billion, which was $3.4 billion more than that of 2010 (NOAA, 2012). Clearly, this sector of the economy is growing rapidly and has become very important.

Aquaculture has the potential to reduce US dependency on imported seafood products and to provide a secure food supply for the global population in the next few decades. However, factors such as biological, technical and economic limitations impact the development of aquaculture. Considering the techno-economic feasibility of aquaculture production, feed cost is a primary concern (Pritchard, 1976). The main source of protein in aquadiets, particularly for carnivorous species, comes largely from ground, ocean caught fishmeal due to its easy availability, high nutritional value and biological qualities in terms of providing required nutrients such as essential amino acids, fatty acids, and digestible energy (ADCP, 1983; FDS, 1994). Approximately two to six pounds of ocean fish are needed for the production of only one pound of farm-raised fish, which results in an average transfer efficiency of 25% (Marine Aquaculture Task Force, 2007.). The increasing rate of global demand for aquaculture products, coupled with the depletion of wild fish stocks has elevated the cost of fishmeal, and consequently, diet prices (Amaya et al., 2007; Lim and Lee, 2009). Therefore, utilization of alternative protein sources can play a crucial role in achieving a sustainable and profitable aquaculture industry (Hardy & Masumoto 1990; Rumsey 1993; Hardy 2010). The goal is to minimize and even to eliminate the fish meal inclusion in aquadiets by substituting appropriate alternative protein sources. To date, many studies have reported the
dietary replacement of fishmeal with different alternative proteins for various species to reduce the diet-cost (Fontainhas-Fernandes et al., 1999; Jauncey, 2000; Coyle et al., 2004; Naylor et al., 2005). Due to its abundance, availability, sustainability, nutrient composition, and relatively low cost, soybean meal is one of the most promising sources of plant-based proteins as a fishmeal replacer in fish diets. Compared with other plant-based protein sources, use of soy products, such as full-fat soybean, toasted defatted soybean meal (SBM), white flakes (WF), and soybean cake in animal and aquaculture feed production are very common. Soybean is rich in both protein and oil. Average protein levels in conventional soybean meal are near 48%, and the average oil levels of defatted soybean meal are approximately 19% (Van Eys et al., 2004); however, variation can occur due to processing variations and the variety of the seed. Additionally, soybean oil is a rich source of ω-3 fatty acids, so can partially be used as a fish oil replacer; soybean oil contains 90% less ω-3 fatty acids than FM (Zhou et al., 2011). Despite their high nutritional value, competing with fish meal protein and essential fatty acids is difficult, as soy products such as soybean meal and soy flour suffer from lack of some essential amino acids (EAA) like lysine and methionine (Wilson, 1989; Floreto et al., 2000), and the presence of antinutritional factors (ANFs) such as trypsin inhibitors, phytates, glycinin, lectin, and non-starch polysaccharides (NSPs).

The oldest commercial fish farming is actually related to trout species farming, out of which rainbow trout (Oncorhynchus mykiss) is one the most common farm raised species in the US (CAES, 1994). It is fairly easy-to-culture carnivorous species that effectively utilizes 25 to 35% fish meal in the diet formulation as the primary protein source (Kim et al., 1991; Hardy, 2002; Cheng and Hardy, 2004). Numerous studies have demonstrated the effect of partial and total soybean meal (SBM) utilization as a dietary protein source for this fish species. Some of these studies have indicated positive performance characteristics with the potential of complete or partially replacement of fishmeal with soy products in fish feeds. The earliest incorporation of soybean meal in trout diets was performed by the Cortland research group in the early 1940s. They suggested that the trout which were fed with soybean meal in their diets had similar growth rates to those fed with meat-meal based diets. Moreover, incorporation of
SBM resulted in a 50% reduction in the trout feed-cost (Tunison et al., 1941). Many studies have shown that FM could be replaced with SBM in rainbow trout diet at the levels between 25 to 80% of the diet (Smith, 1977; Smith et al., 1988; Pongmaneerat and Watanabe, 1992). Olli et al. (1994) studied the effect of four differently processed soy products (solvent-extracted soybean meal, dehulled and solvent-extracted soybean meal, dehulled full-fat soybean meal, and soybean concentrate) on the growth performance of rainbow trout. They replaced high-quality fishmeal at different levels of 14, 28, 42, and 56% with the soybean proteins and concluded that soybean products have the potential to be used in salmonids diets without negative effects due to the ANFs. Kaushik et al. (1995) found that 33 to 100% replacement of fish meal with soy protein concentrate did not affect the growth performance rate of rainbow trout. Also, Gomes et al. (1995) compared the effect of 50% and 100% fish meal replacement with SBM on rainbow trout growth performance with those of two different control diets and suggested that not only the type of alternative protein source was important but the control diet with which the results were compared played a significant role.

However, other research has reported contrasting results with regard to the effect of soy product inclusion on growth performance of aquadiets. For example, Floreto et al. (2000) reported that optimal growth rate for juvenile American lobster (Homarus americanus) could be achieved only with less than 50% replacement of FM with SBM and multiple amino acid supplementations. El-Saidy et al. (2002) studied the potential of complete FM replacement with SBM for Nile tilapia fingerling and revealed that total replacement of FM with 55% SBM and 0.5% lysine supplementation had no adverse effect on growth performance of Nile tilapia. Before them, Dabrowski et al. (1989) also observed a substantial decrease in essential amino acids absorption and growth rate of rainbow trout fed with diets including 25%, 50%, and 100% soybean meal-protein as the fishmeal-protein replacer. Rumsey et al. (1993) explored the inclusion effects of five physiochemically treated soybeans (1-conventional solvent-extracted SBM; 2-special processed solvent-extracted SBM; 3enzyme-treated solvent-extracted SBM; 4-ethanol-extracted solvent-extracted SBM; 5-alkali-treated solvent-extracted SBM) on the growth
performance rate for rainbow trout and suggested that soybean oligosaccharides are required for achieving maximum growth or nitrogen utilization by rainbow trout. For the special processed solvent-extract SBM, they used Aarhus Oliefabrik A/S procedure where the oligosaccharides were not alcohol extracted (Rumsey et al. 1993). Additionally, the protease inhibitor activity of SBM-based fish feed for fingerling channel catfish were studied by Wilson and Poe (1985) and Perse et al. (2003). They reported that by using longer heat treatment SBM could result in less trypsin inhibitor activity (TIA) and greater feed digestibility. Later on, the effect of extrusion processing on two rainbow trout diets including 29% FM -25% SBM and 29%FM-25%WF were examined by Romarheim et al. (2006), who reported a negative influence on growth rate and feed conversion compared with those of FM-based diet. However, TIA in the diets decreased to an appropriate level for rainbow trout species upon extrusion. These contrasting results can be ascribed to factors such as the effect of technological treatments applied to the soybean meal or in general soy protein and the impact of feed production technology on the biological and physical qualities and nutrient availability of the feed. Essential minerals are also very important in balancing the fish metabolism and achieving normal growth (Watanabe et al., 1997). According to Hardy (2002), mineral supplementation of plant-based trout diets is essential.

As mentioned, technological treatments applied to soybeans can influence the biological properties of the feed. Hence, exploring the feasibility of reducing of soybean ANFs has become attractive to the soybean industry in order to promote soy utilization in many feed industries. Soy protein concentrate (SPC) and soy protein isolate (SPI) are thermally and chemically treated SBM which contain nearly 70% and 90% protein (dry basis), respectively. The content of ANFs such as trypsin inhibitors, isoflavones and phytic acids will be changed during the SPC and SPI production processes. In fact, the thermal treatment applied in the SPC inactivates these ANFs, while the inactivation or reduction of the ANFs in SPI is totally dependent on the type of the chemical treatment; furthermore, no thermal treatment is involved in the SPI process.
Fermented soybean meal (FSBM) is a microbial treated form of SBM, and is rich in protein (~56%) and low in ANFs. Antinutritional levels in the FSBM are reduced during the enzymatic degradation and fungal fermentation, and provides many benefits against digestion issues (Kim et al., 1999; Hong et al., 2004). Moreover, the protein quality can be significantly higher than that of SPC due to the low processing temperatures during the microbial process. Over the past decade, there has been a growing interest in evaluating the effects of FSBM inclusion on animal diets, but very little published information addresses the use of FSBM in fish diets (Shimeno et al., 1994; Refstie et al., 2005; Zhou et al., 2011; Yuan et al., 2012). In the last two studies, researchers suggested that FSBM could replace up to 20% and 35% FM in the diets of juvenile Black sea bream, *Acanthopagrus schlegelii* and juvenile Chinese sucker, *Myxocyprinus asiaticus* species, respectively.

Feed production technology also plays a key role in the quality of the aquafeeds. Since the late 1950s and 1960s, extrusion processing has become the most common technology in the aquaculture industry to produce floating feeds (Moscicki and van Zuilichem, 1983). Extrusion is a short-time cooking process, which takes place inside the barrel of the extruder, using moderate to high temperatures, high shear rates, low to moderate moisture contents, and high pressure conditions. Using thermo-mechanical energy input, the raw and semi-dry feed ingredients are mixed, conveyed, transferred, worked into a viscous, plastic-like dough, cooked, and then shaped upon exiting a die restriction, where shaping and expansion phenomena occur due to water latent heat energy and instantaneous changes in water state from liquid to vapor as a result of the pressure release, all of this in a relatively short time (Hardy, 2002). In general, extrusion performance and product quality are influenced by several parameters including feed composition and water content, temperature profile in the barrel sections, screw configuration geometry, die geometry, mass flow rate, residence time, specific mechanical energy, and type of the extruder (Bhattacharya et al., 1997; Chauhan et al., 1998; Sun et al., 2002; Ding et al., 2005; Chevanan et al., 2007a). The interactions among the high shear forces and temperatures, in conjunction with low to moderate moisture inside the extruder influences the ingredient structure at a molecular level, and will
impact the physiochemical, biological, and nutritional properties of the extruded pellets, primarily due to
gelatinization of the starch and denaturation of protein components of the feed blend (Guy, 2001). If
properly controlled, the extrusion cooking process can improve the physical properties of the feed,
inactivate the ANFs, increase the digestibility and bioavailability of nutrients, and reduce the microbial
counts in the feed (Edwards et al, 1994; Steel el al., 1995). Desired physical properties of the extruded
aquafeed include high floatability, water stability, and pellet durability index (Wood, 1995). Appropriate
unit density and expansion ratio of the feed depend on the eating preference of the specific species of fish,
which results in three general categories of feed: floating (i.e. Nile tilapia), slow-sinking (i.e.: rainbow
troll), and rapidly sinking (i.e. catfish). However, in the aquaculture industry, floatable feed is often more
desired, since it can prevent water pollution and nutrient loss (Vens-Cappell, 1984), due to over feeding
High water stability of the extruded feed also plays important role in preventing quick dissolving of the
feed when placed in water, nutrient loss, and thus water contamination. Additionally, the more expanded
extrudates have been shown to have better digestibility, due to the increased gelatinization of starch in the
feed (Ali, 1988; Gokulakrishnana and Bandyopadhyay, 1995; Rout, 1997; Chang and Wang, 1999).
Variation in moisture content of the feed mixture and extrusion processing conditions influence the
expansion, the unit density, and thus the porosity of the extrudates, all of which directly affect the
floatability of the extrudates (Hardy, 2002). Also, the porous texture of expanded extrudates allow for
rapid post-extrusion oil coating and absorption by the extruded pellets (Hardy, 2002).

Typically, twin-screw extruders can have the better performance for production of floating
extrudates in comparison to single screw extruders, since this type of extruder can handle a wider range of
feed blend properties, from low to high moisture, fat, fiber, protein, and starch contents, as well as various
particle size distributions (Riaz, 2000). In addition, positive conveying of the material, better mixing, and
more heat transfer surface area can be obtained in a shorter residence time and at a higher output rate for a
twin screw extruder (Zuilichem and Stolp 1984; Harper, 1989; Shi and Utracki, 1992; Rauwendaal, 2004;
Chevanan et al., 2007b). Moreover, the intermeshing self-wiping property of the twin screws has the advantage of self-cleaning of the machines, resulting in less raw blend waste.

Indubitably, both physical and nutritional qualities of feeds contribute to desired growth performance in the fish, and is achieved as a result of proper processing conditions (Banerjee and Chakraborty, 1998; Lin et al., 1998; Mathew et al., 1999c; Rolfe et al., 2000) in combination with a well-balanced feed formulation (Faubion et al., 1982; Mathew et al., 1999a, b; Cavalcanti and Behnke, 2005a, b). Therefore, the evaluation of extrusion processing impacts on resulting properties of the feed is crucial. For example the effects of extrusion processing on the physical properties of distillers dried grains with solubles-based aquafeeds have been thoroughly examined for some common species, including Nile tilapia and Yellow perch (Chevanan et al., 2007a, 2007b, 2007c, 2008, 2009, 2010; Rosentrater et al., 2009a 2009b; Rosentrater and Tulbek, 2010; Kannadhason et al., 2009a, 2009b, 2010; Ayadi et al., 2011a, b, c, d; Fallahi et al, 2011; Mjoun and Rosentrater, 2011).

To our knowledge, no studies have yet been published specifically focusing on the processing of FSBM-based rainbow trout feeds, neither in evaluating the growth performance nor extrusion processing behavior. Consequently, the goals of the current study were to produce twin-screw extruded feed for juvenile rainbow trout (Oncorhynchus mykiss) using FSBM as an alternative dietary protein source, and to evaluate the effects of FSBM inclusion on the resulting physical properties of the extrudates.

Materials and Methods

Experimental Design and Sample Preparation

Table 1 lists the percent ingredient composition (dry basis percent) for the two experimental diets for rainbow trout and the control feed. Two isocaloric (3.06 kcal/g) experimental diets containing 80 and 100% microbial fermented high protein soybean meal (FSBM), with net protein content of (~39% db), along with appropriate amounts of fish meal (for diet 1 with 80% FSBM), corn gluten meal, wheat flour, vitamin mix, minerals, and essential amino acids, were formulated and were prepared following Ayadi et al. (2011d). FSBM was purchased from NutraFerma (Sioux City, IA); Menhaden fish meal from Omega
Protein Inc. (Houston, TX); 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 and mineral premixes were from Lortscher Agri Service, Inc. (Bern, KS), and essential amino acids from USDA. A control diet containing 100% fish meal as the main dietary protein source was also prepared. Fish meal was reground to a fine particle size of approximately 100 µm with a laboratory-scale grinder (Model S500 disc mill, Genmills, Clifton, NJ), then all ingredients were mixed together 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 (Patterson-Kelly Co. Inc., East Stroudsburg, PA) at 60 rpm for 10 min to produce homogenous blends. The resulting blends were then stored at ambient temperature overnight for moisture content equilibration. The proximate compositions of the diets are given in Table 2.

**Extrusion Processing**

Experimental extrusions were carried out using a semi-industrial twin-screw extruder (Wenger TX-52, Sabetha, KS) with a 30 hp motor and throughput of 50 - 250 kg / h. The extruder was self-wiping with two co-rotating, fully intermeshing screws, a dry feed system, and a continuous preconditioner which was equipped with steam and water injections ports. The dry feed blends were transferred to the feed hopper, which were then conveyed into the preconditioner, where steam was injected at a rate of 0.11-0.16 kg/min. After being adjusted to the desired moisture content and temperature inside the preconditioner, the blends were transferred into the extruder at a feed rate of 20 kg/h, and the conditioner’s screw speed was kept constant. The barrel of the extruder had L/D ratio of 25.5/1, and its twin screws each had a diameter of 52 mm. The screws used in this experiment had 25 individual sections, and the configuration (from the feeding section 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. This configuration was recommended by the manufacturer. Moreover, the barrel was composed of 8 temperature zones, which were set at 15 to 90 ºC. The temperature profile of the barrel varied depending on the actual temperature of each zone and the resulting extrudates properties. The amount of water added to the extruder was maintained at 0.11 to 0.19 kg/min. The extruder had two die nozzles, each with a circular diameter of 3 mm. The exiting extrudates were cut into desired lengths using a rotating three blade cutter mounted at the end of the dies.

**Measurement of Extrusion Processing Parameters**

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).

**Measurement of Extrudate Physical Properties**

The extruded diets were cooled for 72 h at ambient temperature (24±1 ºC) and then dried in a commercial-scale oven (Model TAH-500, Grieve Corporation, Round Lake, IL) for 24 h at 45 ºC. The dried extrudates were then 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 (L*, a*, b*).

**Moisture content (MC):**

MC was determined according to AACC method 44-19 (2000), using a laboratory-scale oven (Fischer Scientific) at 135 ºC for 2h.

**Water activity (a_w):**

Water activity 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.

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

**Expansion ratio (ER):**

The diametral expansion of the extrudates was determined as the ratio of extrudate diameter (mm) to the diameter of the die nozzle (3 mm), using a digital caliper (Digimatic Series No. 293, Mitutoyo Co., Tokyo, Japan.), following Conway and Anderson (1973) and van Zuilichem et al. (1975).

**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, following Jamin and Flores (1998) and Rosentrater et al. (2005). The mass of each of the extrudates was measured with an analytical balance (Adventure 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).

**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 (1/2 L) to the volume of the bulk (cm$^3$) (USDA, 1999).

**Water absorption and water solubility indices (WAI, WSI):**

Following Anderson et al. (1969), using a laboratory-scale grinder (Chemical Rubber Co, CRC, Germany) extrudate samples were ground to a particle size of approximately 150 µm, then 2.5 g of the finely ground sample were placed in a 50 mL centrifuge tube, and 30 mL distilled water at 30 °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 (accuSpin™ 400, Thermo Electron Corporation). Thereafter, the supernatant phase was transferred into aluminum dishes and placed in a laboratory oven (Fisher Scientific) at 135°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):
\[ WSI(-) = \frac{\text{Mass of dry solid}}{\text{Mass of sample}} \]  

(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 the methods of Anderson et al. (1969) and Jones et al. (2000).

2.4.8 Pellet durability index (PDI):

PDI was determined following ASAE standard method S269.4 (ASAE, 1996); 200 g of an 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:

\[ PDI(\%) = \left( \frac{M_a}{M_b} \right) \times 100 \]  

(2)

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), which was calibrated using the standard plates according to the procedure specified by the manufacturer.

Nutrient Analysis

Extrudates were dried at room temperature for 72 hr and were subjected to proximate analysis for protein, fat, fiber, and ash content following the official Methods 990.03, 920.39, 978.10, and 920.48, respectively (AOAC 2003). Each property were measured in duplicate (\( n = 2 \)) for all raw ingredient blends.

Data Analysis
All measurements were made in triplicate, except for UD and ER, where measurements were made 10 times for each run. 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 ($\alpha$) of 0.05, by analysis of variance (ANOVA) to find if there were significant differences among control diet and experimental diets. Then post-hoc LSD tests were used to determine where the specific differences occurred if indeed there were any.

**Results and Discussion**

*Extrusion Processing Parameters*

**Temperature during processing (T):**

Temperatures at three points of the process were monitored. As shown in Table 3, inclusion of FSBM at both levels (80 and 100%) resulted in more than 7% and 4.5% increase in processing temperatures at the conditioner exit ($T_C$) and die exit ($T_d$) compared with those of the control diet, respectively. As expected, the temperature along the extruder barrel length increased proportionally which can be ascribed to heat addition supplied by steam injection, heating barrel temperatures, as well as frictional heating.

*Extrudate Physical Properties*

All results for the physical properties of the extruded products, including moisture content (MC), water activity ($a_w$), thermal properties ($k$, $\alpha$, $R$), expansion ratio (ER), unit density (UD), bulk density (BD), water absorption and water solubility indices (WAI, WSI), pellet durability index (PDI), and color ($L^*$, $a^*$, $b^*$) are provided in Table 4. Considering the main effects of diets on extrudate physical properties, changing the inclusion levels of FSBM exhibited significant effects on almost all of the examined properties, except WSI, $a_w$, and thermal properties.

**Moisture content:**

As shown in Table 4, increasing FSBM content of the blends from 0% to 80% and 0% to 100% resulted in an increase of 15.2% and 22% in the moisture content of the final extrudates, respectively;
though there was no significant change between the raw moisture contents of diet 1 and diet 2 containing 80% and 100% FSBM. In general, moisture contents of the ingredient blend and the final extrudates play important roles in determining the ultimate quality of the products. The plasticizing effect of water influences the flowability (Ganesan et al., 2008) and the rheological properties of the dough; thus resulting changes in the physiochemical quality and the internal structure of the extrudates upon exiting of the die restriction. This mainly occurs due to the interactions among water and other chemical components of the feed blend, and is influenced by the severity of the extrusion processing conditions, especially the high temperatures, shearing forces, and high pressures developed during the process (Miller, 1985; Chevanan et al, 2007b). The internal structure and porosity of the final extrudates are formed due to water evaporation and subsequent expansion of the extruded matrices. Expansion is the product of instantaneous pressure drop and latent heat of vaporization at the die exit (Moore et al., 1990), and is influenced by the extent of gelatinization of the starch and denaturation of the protein components of the melted blend during the process (Nielsen, 1976). The more starch in the blend, potentially the more expansion of the extrudates is possible (Chevanan, 2007b). However, the amount and nature of the starch and protein in the blend are important (Miller 1985; Riaz, 2000; Mercier et al., 1989). The more moisture content of the blend may result in less expansion and higher moisture content of the extrudates, depending upon the water holding capacity of the extrudate’s matrix (Chevanan, 2007b). Therefore, it can impact the cohesiveness of the product, most probably due to the internal liquid-bridge formations among the particles (Johansson, 1978), and consequently can affect the WSI and WAI of the extrudates (Rolfe et al., 2001; Chevanan, 2007b). Additionally, moisture content of the extrudates impacts the storage stability and shelf life of the extrudates (Rolfe et al., 2001), especially the free water which is present.

**Water activity (a_w):**

Water activity of a biological material represents the amount of unbound water (free) that can easily participate in physiochemical reactions, and is freely available to microorganisms. Knowledge about the water activity of a material helps to predict the shelf-life and potential for microbial spoilage.
Also, it can influence the enzyme activity, lipid oxidation, color, and aroma of the biological material. Hence, it is one of the crucial considerations for biological material safety. The strength of the water bound to the other molecules in a material influences the quantity of the $a_w$ of that substance. 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). Therefore, if water molecules are tightly bound to the other molecules in a material, they will not be able to elude from the substance in the form of vapor, and thus no vapor pressure will be released. However, $a_w$ can change depending upon the temperature, since the temperature influences the water binding strength of materials (Higl et al., 2007). In general, the lower water activity of a food or feed material, the less risk of spoilage (Chevanan, 2009).

In this study, inclusion of FSBM did not impact the $a_w$ of the extrudates (Table 4). The average value of the water activity for all the extrudates was less than 0.5 indicating a long shelf-life and low risk of microbial spoilage for the extrudates.

**Thermal Properties:**

As depicted in Table 4, inclusion of FSBM had no significant effect on thermal properties of the extrudates compared to those of the control diet at either level. Knowledge about the relation between the thermal properties of both raw ingredient blend and extruded feed allow us to anticipate the materials’ thermal behaviors, and thus to improve the feed formulation and control the process conditions better in order to achieve a high quality product (Blanche and Sun, 2004). Thermal conductivity of a material indicates its potential for transferring heat through itself due to conduction only, and the driving force is just the temperature gradient. Thermal conductivity of a substance varies depending on its temperature and density; therefore, the required times for post-extrusion drying and cooling processes could be predicted by determining the extrudate thermal conductivity during the heat transfer between the extrudate surface and center, the correspondent temperature difference, and the void spaces of the extrudate (i.e. porosity) (Bouvier and Brisset, 2006). It is expected that with increasing extrudate porosity, the $k$ value of the extrudate decreases, since $k$ of the air trapped inside the extrudate is significantly lower.
than those of the extrudate’s constituents. Thus, the k value of a material is also dependent on its composition. For example, the k value of air is 0.0294 W/m°C at 30°C, while reported values for starch and protein are 0.277 W/m°C, and 0.249 W/m°C, respectively (Choi et al., 1985). It is clear that, the more an extrudate expands, the lower thermal conductivity (Mariam, 2008). In another study, Heldman (2003) postulated that the k value of a heat processed material decreased due to the material changes, primarily because of protein and starch transformations. The presence of hydrophobic constituents in the feed blend affects the density and thus thermal conductivity of the extruded feed (Mariam, 2008).

As per definition, thermal resistivity (R) refers to the ability of a material to prevent heat transfer through that material. Thermal resistivity of material varies as a function of material thickness and the temperature gradient across the material, which makes it a time dependent parameter (Arambula-Villa et al., 2007). According to Kawasaki and Kawai (2006), thermal diffusivity (α) is ascribed to the material’s capability to store the heat vs. heat transfer. Being low heat conductive and high heat resistant, the raw blend may require longer time for cooking, while the blends with low heat conductivity and diffusivity are less affected by external thermal sources and may be more stable (Ayadi et al. 2011b). A positive relation between the expansion ratio and α value of a complete vegetable-based yellow perch diet was observed in an unpublished twin-screw extrusion study carried out by our research group, which could be attributed to the higher porosity of the more expanded extrudates. The thermal diffusivity of the air is larger than those of the protein and starch by a double order of magnitude (Mariam et al., 2008). Hence, thermal properties of the extrudates are highly related to diet formulation, extrusion operational conditions, and internal structures 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.

**Expansion ratio (ER):**

The resulting data revealed that FSBM incorporation increased the ER value of the extrudates compared to that of the control diet. Inclusion of 80% and 100% FSBM increased the ER by 5% and
10%, respectively, although changing FSBM incorporation level had no statistically significant effect on the extrudate’s ER (Table 4). ER is one of the most important quality parameters for fish feed extrudates. Expansion is mainly governed by the sudden decrease in pressure at the die section during the evaporation phenomenon, which leads to water phase transition and formation of air cells in the extrudates (Alves et al. 1999; Lam and Flores 2003; Moore, 1990; Chevanan et al., 2007a), and is directly related to the extrudate buoyancy and brittleness (Rosentrater et al., 2009a, b; Rolfe et al., 2001). The magnitude of extrudate ER is influenced by several factors such as the dough moisture content, die dimensions, feed ingredient conditions (Tumuluru and Sokhansanj, 2008; Bouzaza et al., 1996; Chevanan et al., 2007a) mass flow rate (Tumuluru and Sokhansanj, 2008; Chevanan et al., 2007a), residence time in the extruder, and rheological behavior of the dough (Fan et al., 1994; Mitchell et al., 1994; Chevanan et al., 2007a). The pseudoplastic behavior of the melted dough in the extruder will result in a substantial decrease in the apparent viscosity of the dough and ultimately will affect the extent of pressure release and expansion of the finished product (Rosentrater et al., 2005; Chevanan et al., 2007a). Basically, the more water present in the blend can cause more viscosity reduction, it can reduce friction between the screw and the barrel, it can decrease pressure inside the die, it also facilitates the melted dough flow through the die section, and thus reduces the extent of extrudate expansion primarily due to the lubricating effect of water (Mjoun and Rosentrater, 2011). Higher starch content causes more extrudate expansion due to starch gelatinization and elastic behaviour of the dough inside the barrel and subsequent pressure rise at the die zone (Nielsen, 1976; Sokhey et al., 1994; Ibanoglu et al., 1996; Lin et al., 2000). Various starch sources may exhibit different effects on the extruded fish feeds depending upon the molecular structure and type of the starch and proteins present in the feed (Kannadhason et al., 2009a). A positive effect of barrel temperature on extrudate ER was observed by Kim et al (1989) who recommended that higher temperature caused more starch gelatinization as well as super-heated steam excretion and consequently more expansion. A higher ER can improve the feed digestibility (Rout, 1997). Where extrusion of starchy blends results in more expanded products, extrusion of proteinaceous blends
leads to production of more porous extrudates due to the protein denaturation (Singh et al 1991; Sandra and Jose 1993; Chevanan et al., 2007a).

**Unit density (UD):**

Unit density is another crucial property of the feed which is inversely related to the ER, and it impacts the floatability of the extruded feeds (Oliveira et al., 1992). As shown in Table 4, FSBM inclusion decreased the UD of the extrudates curvilinearly. Total replacement of FM with FSBM (i.e. diet 2) decreased the UD by nearly 9.5%, while partial replacement of FM resulted in a 6.8% decrease in UD. However, changing levels of FSBM inclusion from 80% to 100% did not exhibit statistically significant effect on the UD of the extrudates. FSBM inclusion at both levels resulted in buoyant products with UD values of less than that of water (1.0). As was expected, UD and ER values were inversely related (Bhatnagar and Hanna, 1986; Colonna et al., 1989,) and all the extrudates did float. Extrusion conditions and interactive effects of several correspondent variables can influence extrudate UD values. For example, Tumuluru and Sokhansanj (2008) proposed that MC of the feed blend contributed not only to binding gelatinized starch, denatured protein and other ingredients of the diet but also toward the unit density of the extrudates. They also observed that barrel temperature, screw speed, and die L/D could impact the UD values of the extrudates. In an earlier research work conducted by Chevanan et al. (2007a), they postulated that MC did not show any significant effect on the UD values of the extrudates; however, barrel temperature showed negative effect on extrudate UD in their study. Their results were in agreement with what Bhattacharya and Hanna (1986) reported. They recommended that increasing barrel temperature progressively reduced the UD, which could be attributed to the shear thinning effect, higher temperatures and a subsequent increase in ER, and thus a decrease in UD of the extrudate. The amount of conditioner steam and extruder water added during the extrusion process to adjust the MC of the feed are also factors affect UD values (Rosentrater and Tulbek, 2010; Fallahi et al., 2011).

**Bulk density (BD):**
Bulk density of any kind of processed materials (including biological products like fish feed) plays a vital role in cost estimation of the product. Transport and storage costs, decisive factors in product final cost, are a function of bulk density, which varies with size, shape, and ER of the extrudates (Chevanan et al., 2007a), and thus can strongly affect the required storage space (Guy, 2001; Rosentrater 2006), and transportation expense. Clearly, the higher the bulk density, the lower the packaging, storage and transportation costs. As shown in Table 4, increasing levels of FSBM incorporation decreased the BD of the extrudates. Using 80% and 100% FSBM as the FM replacer led to a 5% and 7.3% decrease in the BD of the product, respectively. Changing FSBM inclusion level from 80% to 100% resulted in 2.5% decrease in BD. Our observations for ER and UD of the extrudates confirm the direct and inverse relation of BD to UD and ER, respectively. The highest floatability was observed for extrudates produced from the complete FSBM-based diet (i.e. diet 2) which had the highest ER, lowest UD and BD of 1.30 (-), 658.91 (g/cm3), and 530.16(g/cm3), respectively.

**Water absorption index (WAI):**

Table 4 shows that FSBM inclusion at both levels resulted in a progressively decrease in the WAI. Inclusion of 80% FSBM led to a 17.24% decrease in WAI; further increasing FSBM to 100% reduced the WAI by 27.6%. Looking at the data presented for ER (Table 4), there was an inverse relationship between the ER and WAI values of the extrudates, indicating that the more expanded extrudates absorbed less water. Our observations were in contrast with what Adeparusi and Famurewa (2011) reported. Basically, WAI implies the amount of water absorbed by the starch components of the extrudates which were not affected by extrusion processing and thus retained their native structures (Mason and Hoseney, 1986; Govindasamy, 1996; Chevanan et al., 2007a). Mjoun and Rosentrater (2011) indicated that WAI of the feed blends, where the starch was not the only component, indicated the water holding capacity of the biopolymers present in the blend. Other researches recommended that variation in the WAI values reflects the structural modification of the blend constituents, mainly due to starch gelatinization and protein denaturation (Badrie and Mellowes, 1991; Chevanan et al., 2007a; Rosentrater
et al., 2009a, b). Apparently, the greater the WAI of the extrudates can be attributed to less molecular weight reduction of amylose and amylopectin molecules during extrusion processing.

**Water solubility index (WSI):**

While WAI was related to the quantity of the undamaged portion of the starch component of the ingredient blend, WSI directly refers to the extent of transformed starch (Harper 1981) and denatured protein (Colonna and Mercier, 1983) during extrusion processing. It has been shown that temperature elevation during extrusion processing increases WSI as a result of starch depolymerization (Anderson et al 1982). As mentioned earlier, depolymerization primarily happens due to reduction of amylose and amylopectin length. Generally, depolymerization of the macromolecules is strongly influenced by the combination effects of temperature, pressure, and shear forces during the extrusion processing which increases the WSI (Menegassi et al., 2011; Anderson et al., 1982) as well as plasticization by water. In our experiments, as FSBM increased, WSI increased curvilinearly. Inclusion of 80% FSBM led to a significant increase in WSI by 23.7% compared to that of the control diet; further increasing of FSBM to 100% reduced and increased the WSI by 10% and 11% compared with those of diet 1 and the control diet, respectively. However, there was no statistically significant difference between the WSI values of diet 1 and diet 2. Furthermore, there was an inverse relationship between WAI and WSI values of the extrudates (Anderson et al., 1982).

**Pellet durability index (PDI):**

Durability of the extrudates after the manufacturing also needs to be considered. Generally, aquafeed extrudates are transported and stored in large bags or bins until feeding time, when the feed distribution can be performed either manually or mechanically. Therefore, the resistance of the extrudates against abrasion and breakage is very important in order to avoid product wastage, nutrient loss, water pollution, and subsequent economic losses. In other words, pellet durability index quantifies the mechanical strength of extruded products against the external forces. Indeed, intensity of the heat transfer and structural alteration of starch (at molecular level), influenced by the synergistic effects of barrel
temperature and feed blend water affect the durability of extrudates (Colonna et al., 1989; Chevanan et al., 2007a; Rosentrater et al., 2009a, b). As shown in Table 4, FSBM incorporation significantly increased the PDI values of the extrudates. This increasing PDI trend may be attributed to the FSBM protein characteristic. The structural alteration of FSBM protein during the extrusion process could affect water distribution in the produced matrix, and consequently influence the expansion and binding properties of the extrudates (Bhattacharya, 1997; Bhattacharya et al., 1986; Fernandez-Gutierrez et al., 2004). All extrudates had PDI values of higher than 99.5%, implying the excellent durability.

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

The effects of each diet on color of the extrudates are provided in Table 4. Inclusion of FSBM in the rainbow trout diet at both levels of 80% and 100% significantly increased a* and b* values of the extrudates by 36%, 73%, 10%, and 30% compared with those of the control diet, respectively; only total replacement of FM with FSBM increased the L* value of the extrudate (by 12.52%).

Color is an important visual quality of biological products (Ilo and Berghofer, 1999). Changes in color of food products during thermal processing can be attributed to the effect of Maillard reactions (Mercier et al., 1989) which reduces lysine availability of the protein-based products through the blockage of basic essential amino acids, mainly lysine, and formation of amadori lysine complexes such as furosine, leading to lysine unavailability (Finot, 1982; Bjorck and Asp 1983; Bjorck et., 1985). Maillard reactions occurring during the extrusion processing can destroy amino acid chains of the protein molecules (Rosentrater et al., 2005; Dahl and Villota, 1991), and thus decrease the protein digestibility. Chevanan et al (2007a) who claimed that changing in color of extruded products compare to raw blends could be considered as a sign of lysine loss or alteration.

**Conclusions**

Increasing levels of microbial fermented soybean meal (FSBM) from 0% to 100% in rainbow trout feed resulted in more expanded extrudates. FSBM incorporation increased WSI values curvilinearly and decreased the BD of the extrudates. All the extrudates had excellent durability, floatability, and
storage stability. Overall FSBM is a promising protein alternative for the aquaculture industry. Yet, more research needs to be done to examine the effect of extrusion processing parameters on properties of the FSBM-based diets as well as nutritional efficacy by conducting feeding trials.

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http://dx.doi.org/10.111/j.17497345.2009.00328.x.


http://dx.doi.org/10.1007/s10086-005-0720-0.


http://dx.doi.org/10.1038/35020537


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

<table>
<thead>
<tr>
<th>Components (%db)</th>
<th>Control</th>
<th>Diet 1</th>
<th>Diet 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry weight of ingredients (g/100g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PepSoyGen(^a)</td>
<td>0.00</td>
<td>47.20</td>
<td>58.84</td>
</tr>
<tr>
<td>Fish meal(^b)</td>
<td>45.90</td>
<td>11.54</td>
<td>0.00</td>
</tr>
<tr>
<td>Corn gluten meal(^c)</td>
<td>28.07</td>
<td>23.72</td>
<td>22.53</td>
</tr>
<tr>
<td>Whole wheat flour(^d)</td>
<td>22.47</td>
<td>10.17</td>
<td>11.27</td>
</tr>
<tr>
<td>CMC(^e)</td>
<td>0.76</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Vitamin premix(^f)</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Mineral mix(^g)</td>
<td>0.00</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Oils</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Supplements (total from below)

<table>
<thead>
<tr>
<th>Supplements</th>
<th>Control</th>
<th>Diet 1</th>
<th>Diet 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stay-C</td>
<td>0.63</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Choline</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Phytase</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>DV Aqua</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.00</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.63</td>
<td>1.14</td>
<td>1.14</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.76</td>
<td>0.89</td>
<td>0.88</td>
</tr>
<tr>
<td>Manganese oxide</td>
<td>0.00</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>0.00</td>
<td>2.91</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Total: 100.00 100.00 100.00

\(^a\) PepSoyGen, NutraFerma (Sioux City, IA)
\(^b\) Menhaden fish meal, Omega Protein Inc (Houston, TX).
\(^c\) Corn gluten meal, Consumers Supply Distributing Company (Sioux City, IA)
\(^d\) Whole wheat flour, Bob’s Red Mill Natural Foods, Inc. (Milwaukie, OR)
\(^e\) Carboxyl methyl cellulose (CMC), USB Corporation (Cleveland, OH)
\(^f\) Vitamin premix, Lortscher Agri Service, Inc. (Bern, KS)
\(^g\) Mineral, Lortscher Agri Service, Inc. (Bern, KS)
<table>
<thead>
<tr>
<th></th>
<th>1st Diet (%)</th>
<th>2nd Diet (%)</th>
<th>3rd Diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein</td>
<td>39.96</td>
<td>39.51</td>
<td>38.15</td>
</tr>
<tr>
<td>Crude Fat</td>
<td>16</td>
<td>15.99</td>
<td>15.98</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>0.36</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>Ash</td>
<td>2.03</td>
<td>4.93</td>
<td>4.93</td>
</tr>
</tbody>
</table>

**TABLE 2.** Proximate compositions of the diets (%db)
## TABLE 3. Treatment effects on extrusion processing parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Diet 1</th>
<th>Diet 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing temperature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeder zone</td>
<td>25.44&lt;sup&gt;b&lt;/sup&gt; (0.20)</td>
<td>25.7&lt;sup&gt;b&lt;/sup&gt; (0.22)</td>
<td>25.78&lt;sup&gt;a&lt;/sup&gt; (0.18)</td>
</tr>
<tr>
<td>Conditioner zone</td>
<td>30.76&lt;sup&gt;b&lt;/sup&gt; (0.53)</td>
<td>33.01&lt;sup&gt;a&lt;/sup&gt; (0.94)</td>
<td>33.15&lt;sup&gt;a&lt;/sup&gt; (1.19)</td>
</tr>
<tr>
<td>Die zone</td>
<td>46.02&lt;sup&gt;b&lt;/sup&gt; (1.03)</td>
<td>48.07&lt;sup&gt;a&lt;/sup&gt; (1.11)</td>
<td>48.24&lt;sup&gt;a&lt;/sup&gt; (1.22)</td>
</tr>
</tbody>
</table>

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.
TABLE 4. Main effects on extrudate physical properties.

<table>
<thead>
<tr>
<th>Property</th>
<th>Control</th>
<th>Diet1</th>
<th>Diet2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC (%)</td>
<td>8.32(^b)</td>
<td>9.81(^a)</td>
<td>10.18(^a)</td>
</tr>
<tr>
<td></td>
<td>(0.12)</td>
<td>(0.79)</td>
<td>(0.79)</td>
</tr>
<tr>
<td>(a_w) (-)</td>
<td>0.48(^a)</td>
<td>0.49(^a)</td>
<td>0.49(^a)</td>
</tr>
<tr>
<td></td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
</tr>
<tr>
<td>(k) (W/(m.C))</td>
<td>0.06(^a)</td>
<td>0.06(^a)</td>
<td>0.052(^a)</td>
</tr>
<tr>
<td></td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>(R) (m.C /W)</td>
<td>18.47(^a)</td>
<td>17.92(^a)</td>
<td>18.78(^a)</td>
</tr>
<tr>
<td></td>
<td>(0.90)</td>
<td>(1.04)</td>
<td>(0.74)</td>
</tr>
<tr>
<td>(\alpha) (mm(^2)/s)</td>
<td>0.16(^a)</td>
<td>0.16(^a)</td>
<td>0.17(^a)</td>
</tr>
<tr>
<td></td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
</tr>
<tr>
<td>ER (-)</td>
<td>1.18(^b)</td>
<td>1.24(^a)</td>
<td>1.30(^a)</td>
</tr>
<tr>
<td></td>
<td>(0.08)</td>
<td>(0.04)</td>
<td>(0.07)</td>
</tr>
<tr>
<td>UD (kg/m(^3))</td>
<td>731.70(^a)</td>
<td>682.105(^b)</td>
<td>658.91(^b)</td>
</tr>
<tr>
<td></td>
<td>(100.11)</td>
<td>(46.75)</td>
<td>(67.93)</td>
</tr>
<tr>
<td>BD (kg/m(^3))</td>
<td>572.15(^a)</td>
<td>544.02(^b)</td>
<td>530.16(^c)</td>
</tr>
<tr>
<td></td>
<td>(1.30)</td>
<td>(8.49)</td>
<td>(2.49)</td>
</tr>
<tr>
<td>WAI (-)</td>
<td>3.48(^a)</td>
<td>2.88(^b)</td>
<td>2.52(^c)</td>
</tr>
<tr>
<td></td>
<td>(0.06)</td>
<td>(0.22)</td>
<td>(0.12)</td>
</tr>
<tr>
<td>WSI (%)</td>
<td>14.81(^b)</td>
<td>18.32(^a)</td>
<td>16.46(^ab)</td>
</tr>
<tr>
<td></td>
<td>(0.24)</td>
<td>(3.04)</td>
<td>(0.73)</td>
</tr>
<tr>
<td>PDI (%)</td>
<td>99.46(^c)</td>
<td>99.59(^b)</td>
<td>99.84(^a)</td>
</tr>
<tr>
<td></td>
<td>(0.05)</td>
<td>(0.08)</td>
<td>(0.07)</td>
</tr>
<tr>
<td>L* (-)</td>
<td>22.36(^b)</td>
<td>22.56(^b)</td>
<td>25.16(^a)</td>
</tr>
<tr>
<td></td>
<td>(0.21)</td>
<td>(0.59)</td>
<td>(0.46)</td>
</tr>
<tr>
<td>a* (-)</td>
<td>5.51(^c)</td>
<td>7.52(^b)</td>
<td>9.55(^a)</td>
</tr>
<tr>
<td></td>
<td>(0.10)</td>
<td>(0.26)</td>
<td>(0.31)</td>
</tr>
<tr>
<td>b* (-)</td>
<td>9.00(^c)</td>
<td>9.91(^b)</td>
<td>11.72(^a)</td>
</tr>
<tr>
<td></td>
<td>(0.21)</td>
<td>(0.27)</td>
<td>(0.30)</td>
</tr>
</tbody>
</table>

MC is moisture content; \(a_w\) is water activity; \(K\) is thermal conductivity; \(R\) is thermal resistivity; \(\alpha\) 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; Parentheses indicate ±1 standard deviation; Means followed by similar letters for a given dependent variable are not significantly different at \(P<0.05\) among treatments.