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Extruded Aquaculture Feed: A Review

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Abstract

Agro-industrial by-products are processed materials that can have high protein content or other nutrients. The agro-industrial by-products are traditionally sold at low prices for animal feed consumption. These residues of the agro-industry have a high concentration of nutritional and bioactive compounds, which can be applied as fishmeal substitutes. In this chapter, it is shown how extrusion can be an alternative process for aquaculture feed production, increasing digestibility, and functional properties of the aquaculture feed, such as water stability and floatability. The thermal process during extrusion decreases the antinutritional factors present in legumes or other agro-industrial by-products, such as trypsin inhibitors and lectins. This chapter reviews research related to new protein sources that can potentially complement or substitute fishmeal for aquaculture feed. The use of bean (*Phaseolus vulgaris*) protein and cottonseed meal as a fishmeal substitute are shown, as well as the optimization of the extrusion process for aquaculture feed production. The incorporation of plant protein into the aquaculture production contributes to a more sustainable process. The effect of the extrusion parameters on the final product and quality are explained.

Keywords: cottonseed meal, legumes, functional properties, fishmeal, extrusion, aquaculture

1. Introduction

The Food and Agriculture Organization (FAO) considers that about 16% of the consumed animal protein comes from fish proteins [1]. With increasing population, demand for fish consumption will increase. Aquaculture is a good alternative for wild fish production. Aquaculture is a growing economic activity with estimated sales in the USA in 2013 of \$1.3 billion dollars [2]. About 43% of the world's fish production has increased in farms and has been increasing in the past decade, especially in Asia and Africa [3]. Worldwide the aquaculture production

grew in 2013 to 97.2 million tons (live weight), with a value of 157 billion US dollars. Asia is the major aquaculture producer in the world. Aquaculture production, such as that of finfish and crustaceans, requires a high amount of fishmeal [1]. Fishmeal represents about 60% of the cost of aquaculture feed and is also a limited resource. The world market consumes about 68% of fishmeal for aquaculture products, such as shrimp, trout, salmon, and other species [4], and it is expected to grow in the next decades. Several investigations have looked into the use of alternative protein sources that could either supplement or substitute for fishmeal. Agro-industrial by-products have been successfully used for animal feed [5], but can also be applied in aquaculture.

Soybean has been successful to a certain point as a substitute for fishmeal as a protein source for different aquaculture species. Reports show the use of soybean in feeding trout and shrimp. About 47% of world soybean production is GMO-soy [6]. Up to 69% soybean in the global market is genetically modified, while 85% of the soy produced in the USA is genetically modified. Consumers are also looking for nonGMOs for consumption. Different legumes represent an alternative source of protein usable for aquaculture feed. Agro-industrial by-products have high protein concentration and are sold at low cost for animal feed. Bean (*Phaseolus vulgaris*) is a common legume grown and consumed in many countries and different climates. Bean plants have low water requirements and are a staple food in many areas of the world. Small and damaged beans have no economic value and represent an agricultural by-product. Beans are an excellent protein source for aquaculture feed if processed thermally to inactivate the antinutritional factors present in the kernel [7, 8]. Cottonseed meal is a by-product of the oil industry. After oil extraction, the cottonseed meal (CSM) can have a protein content of up to 55%. CSM is sold at low prices for cattle feed and other small ruminants. The presence of gossypol, an antinutritional agent in CSM, limits its use in aquaculture. The breeding program has developed cotton varieties with low gossypol content, acceptable for use in aquaculture [9]. Some aquaculture species have low amylases activity, which limits the enzymatic breakdown of starches.

Extrusion applies high-temperature in short processing times. Extrusion is an alternative for feed production, increasing digestibility, and functional properties of the aquaculture feed, such as water stability and floatability. The thermal process during extrusion decreases the antinutritional factors present in legumes or other agro-industrial by-products, such as trypsin inhibitors and lectins [8]. The chapter reviews agro-industrial by-products than can substitute or complement fishmeal for aquaculture feed. The optimization of the extrusion process for aquaculture feed production is discussed.

2. Chemical composition and diet requirements in extruded aquaculture products

Agro-industrial by-products are processed materials, where some of the main compounds have been either extracted or are products that do not comply with certain quality requirements. The oil extraction industry produces by-products with high protein, while distillery by-products have less sugar after fermentation, but high protein concentrations. The bean (*P. vulgaris*) processing industry discards small kernels with no commercial application.

The agro-industrial by-products are traditionally sold at low prices for animal feed consumption. These residues of the agro-industry have a higher concentration of nutritional and bio-active compounds, which can be either used as supplements in functional foods or extracted and used for nutraceuticals. After oil extraction, soybean meal, canola meal, and flax seed meal contain high concentrations of proteins, minerals, and fiber. The brewing and distillery industry also offers by-products with high protein concentration. Cottonseed meal is obtained after oil extraction from the cottonseed. Glandless cottonseed has a low concentration of gossypol and is suitable for consumption by humans as well as livestock and aquaculture products. The levels of gossypol present in glandless cottonseed meal are not toxic for monogastric animals. The concentration of protein in glandless cottonseed meal (GCSM) can be up to 55%, which is more than 100% protein increased, compared to the cottonseed. GCSM has more protein than canola meal and is comparable to soybean meal (**Table 1**). Soybean seeds can have 40% of protein [10], but its content increases after oil extraction. Cottonseed meal has low starch content and a high mineral content, which makes it an excellent complement for aquaculture feed. Small and cracked beans do not have the required quality for consumer acceptance, but have a high protein and starch content (**Table 1**). Although the protein content is lower than other agro-industrial by-products presented in **Table 1**, the high starch content makes it more suitable for extrusion than by-products with low starch content. The extruded bean flour can be used for human and animal consumption, as well as for the aquaculture feed industry. The extrusion process totally inactivates the trypsin inhibitors and lectins in the bean flour [8].

Legumes have high protein content, and can potentially substitute for fishmeal. Bean flour can substitute for fishmeal in aquaculture feed. A balanced aquaculture feed should contain

| Product | Fat (%) | Crude protein (%) | NFE (%) | Mineral content (%) | Crude fiber (%) |
|--|---------|--------------------------|---------|---------------------|-----------------|
| Soybean meal ⁴ | 1.59 | 48.3 | 40.1 | 6.53 | 3.5 |
| Canola meal ⁴ | 2.8 | 36.1 | 43.3 | 6.3 | 11.5 |
| Flax seed meal ⁴ | 2.2 | 38.9 | 46.6 | 7.0 | 5.3 |
| DDGS ⁵ | 10.9 | 30.2 | 53.1 | 5.8 | 8.8 |
| Cotton seed ³ | 20.71 | 23.1 | 31.8 | 4.85 | 19.54 |
| Cottonseed meal ² | 12.6 | 41.0 ³ , 55.3 | 8.2 | 7.8 | 1.0 |
| Bean (<i>P. vulgaris</i>) ⁶ | 1.8 | 18.6 | 51.9** | 3.7 | 11.0 |

¹Solvent extracted.

²Cold pressed (Reyes-Jaquez et al. [11]), Gerzhova et al. [12].

³Gui et al. [13], Mujahid et al. [14].

⁴Khattab and Arntfield [15].

⁵Spiehs et al. [16].

⁶Delgado et al. [8].

*Total carbohydrates, Distiller's dried grains.

**Total starch content.

Table 1. Comparison of chemical composition of different agro-industrial by-products.

proteins and essential fatty acid, normally from fish oil, minerals, and vitamins. Aquaculture feed contains about 62% fishmeal, 20% wheat flour, 20% fish oil, 3.4% milk whey, 2.1% vitamins and minerals, and 0.5% choline chloride for cell function and structure (Table 2). The use of plant proteins should supply enough proteins to cover the nutritional requirements of the aquaculture products. Fishmeal is a limited resource, but alternative protein sources can substitute for fishmeal in aquaculture diets.

| Composition | Fish meal | Bean flour concentration | | | Soy protein concentrate | | |
|-------------------------|-----------|--------------------------|------|------|-------------------------|------|------|
| | | 15% | 30% | 45% | 15% | 30% | 45% |
| Fishmeal | 62.0 | 52.7 | 43.4 | 34.1 | 52.7 | 43.4 | 34.1 |
| Bean flour | – | 9.3 | 18.6 | 27.9 | – | – | – |
| Soy protein concentrate | – | – | – | – | 9.3 | 18.6 | 27.9 |
| Wheat flour | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Fish oil3 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 |
| Dried whey | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 | 3.4 |
| Choline chloride | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Vitamin and minerals | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Chromic oxide | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

*Rodríguez-Miranda et al. [17].

Table 2. Chemical composition of aquaculture diets (%)*.

3. Hardness and functional properties of extruded products

Hardness, Water Absorption Index, and Water Solubility Index are essential functional properties of aquaculture feed. The feed should have a certain hardness for the trout or shrimp to be able to eat it. The hardness of the extruded product depends on extrusion moisture and extrusion temperature. High extrusion temperature and high moisture content result in a hard extruded product. Softer products are the result of extruding at low temperatures and high moisture content (Figure 1). Aquaculture feed products need a specific Water Absorption Index (WAI) to facilitate consumption, while the Water Solubility Index (WSI) correlates well with the stability of the feed in an aqueous environment. The extruded products need to be stable in the water; a high WAI also produces a high WSI of the extrudates. Studies show that extruded products with lower WSI are obtained at low extrusion temperatures and low moisture content, which also produces harder extruded products. If we compare Figures 1 and 2, we can conclude that although crystallinity is lower in the product extruded at higher temperatures, the hardness tends to be high. The results indicate a probable high degree of denaturation, where proteins unfold, allowing protein to restructure into harder structure.

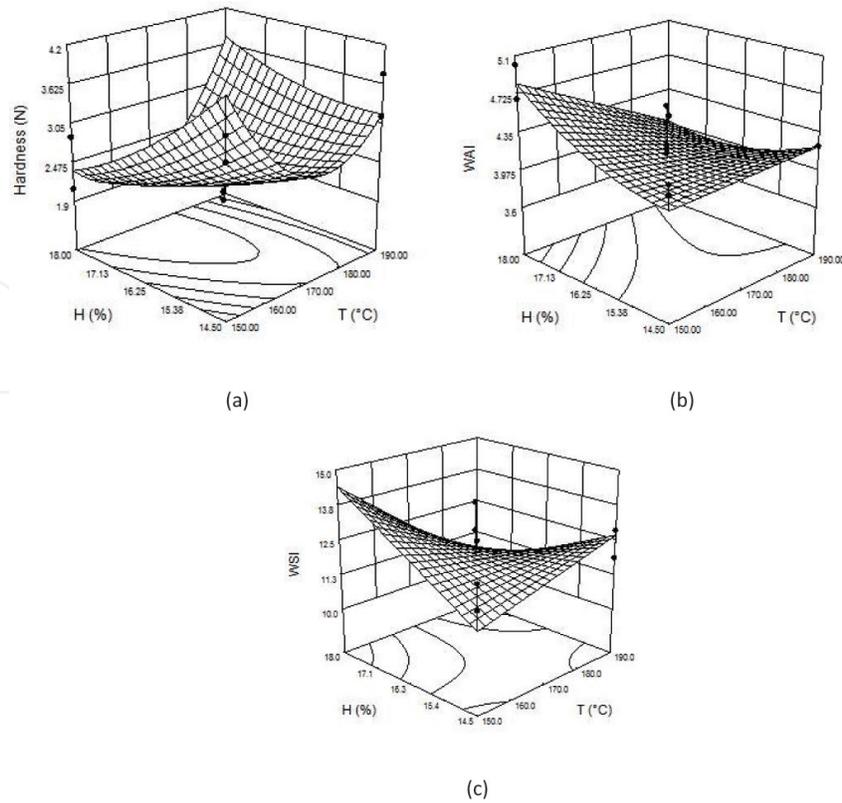


Figure 1. Effect of temperature and moisture content on the (a) hardness, (b) Water Absorption Index (WAI), and (c) Water Solubility Index (WSI) of extruded products from bean-nixtamalized corn flour. Design points above or below predicted value (reproduced with permission from Atienzo-Lazos et al. [7]).

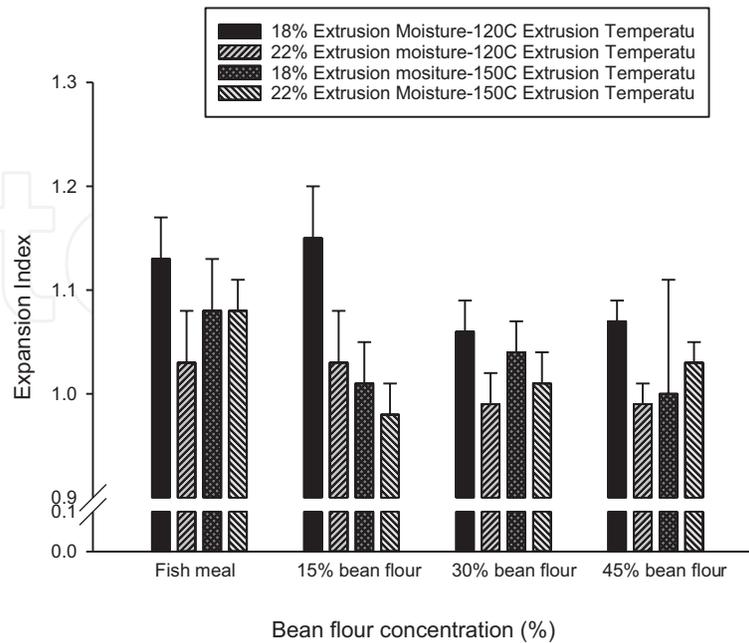


Figure 2. Expansion Index of extruded aquaculture feed at different extrusion moisture with fishmeal and different concentrations of bean flour (*P. vulgaris*). Vertical bars indicate standard deviation of the mean (Rodríguez-Miranda et al., 2014).

4. Extruded bean flour

Studies show that with a fishmeal substitution of 15, 30 and 45% bean flour or soy protein, there are no significant ($p > 0.05$) changes in protein content (**Table 3**). Even a 45% fishmeal substitution with bean flour/soy protein had similar protein content as the diet with no vegetable protein. The fat content of the aquaculture feed ranged between 15.9 and 18.8%, and the dry matter content was 91.6% or higher. The mineral content was significantly lower in the diets with 45% bean flour and with soy protein, compared to the diet with only fishmeal (**Table 3**). Bean flour is a better source of minerals for fish diet than soy protein concentrates. The functional properties of the extruded products are affected ($p < 0.05$) by the substitution of fishmeal with vegetable proteins. The Expansion Index (EI) decreases depending on the extrusion moisture. The EI decreases when substituting 30 and 45% of the fishmeal with bean flour at 18 and 22% extrusion moisture (**Figure 2**). When extruding at 22% moisture the EI and the bulk density (BD) were not affected ($p < 0.05$) by the substitution of fishmeal by bean flour (**Figure 3**) [18]. The Water Absorption Index (WAI) is also affected ($p < 0.05$) by the presence of bean flour (**Figure 4**). An increase in bean flour and decrease in fishmeal decrease ($p < 0.05$) the WAI and increase ($p < 0.05$) the Water Solubility Index (WSI) at 22% extrusion moisture. The WSI does not change ($p > 0.05$) at 18% extrusion moisture, and it even decreases at 22% extrusion moisture. The stability of the extrudates depends on the solubility and water absorption indices. The lower the WSI, the more stable the feed will be (**Figure 5**).

Another quality parameter in aquaculture feed is the sinking velocity of the product. The sinking velocity of the aquaculture feed is different for each aquaculture species. Some fish requires slow sinking feed that will resemble the movement of small fish or other living organisms. In the case of shrimp, the feed should sink to the bottom of the ponds for better use. Extrusion temperature affects ($p < 0.05$) the sinking velocity of the feed (**Figure 6**). Extrusion at 120°C increases ($p < 0.05$)

| Extruded diet | Fat (%) | Crude protein (%) | NFE* (%) | Mineral content (%) | Dry matter (%) |
|-----------------------------------|--------------------|-------------------|-------------------|---------------------|--------------------|
| With fish meal ¹ | 16.9 ^{bc} | 48.7 ^a | 29.2 ^a | 12.9 ^b | 91.7 ^b |
| With 15% bean flour ¹ | 18.8 ^d | 41.9 ^a | 27.8 ^a | 11.4 ^b | 91.6 ^b |
| With 30% bean flour ¹ | 16.7 ^{ab} | 40.9 ^a | 24.1 ^a | 10.4 ^b | 91.9 ^{bc} |
| With 45% bean flour ¹ | 16.4 ^a | 38.7 ^a | 35.0 ^a | 9.7 ^a | 92.5 ^b |
| With 15% soy protein ² | 17.7 ^c | 40.9 ^a | 32.4 ^a | 8.9 ^a | 92.6 ^a |
| With 30% soy protein ² | 15.9 ^a | 42.2 ^a | 32.2 ^a | 9.5 ^a | 92.4 ^{ac} |
| With 45% soy protein ² | 16.0 ^a | 45.3 ^a | 29.5 ^a | 91.9 ^a | 92.3 ^{ac} |

¹Rodríguez-Miranda et al. [17, 18].

^{a-c}Values with different letters in the same column are significantly different ($p < 0.05$).

*NFE = Nitrogen free extract.

Table 3. Chemical composition of extruded aquaculture feed with fishmeal and different concentrations of bean flour (*Phaseolus vulgaris*).

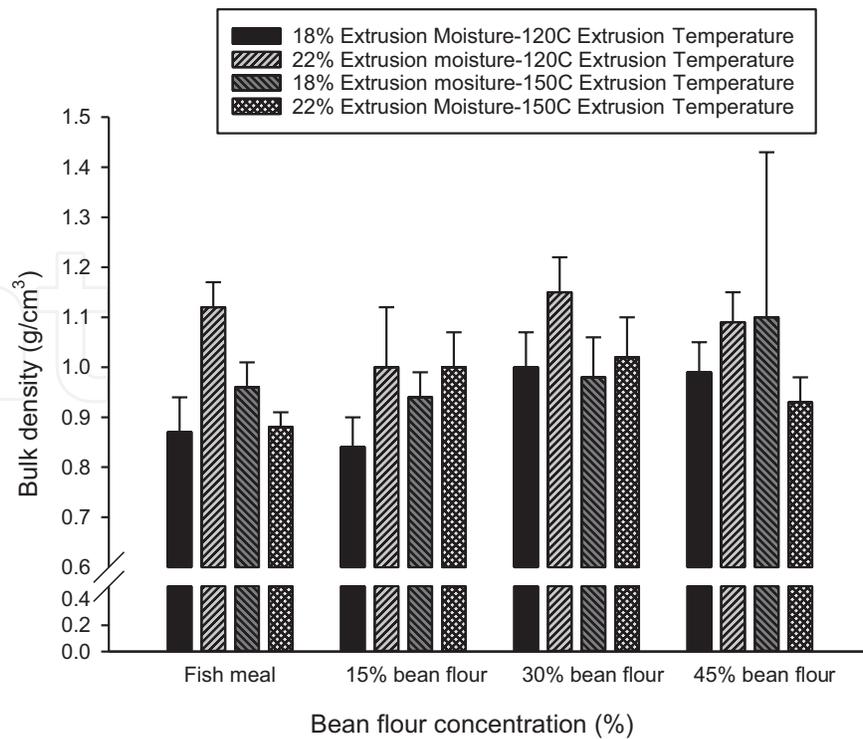


Figure 3. Bulk density of extruded aquaculture feed at different extrusion moisture with fishmeal and different concentrations of bean flour (*P. vulgaris*). Vertical bars indicate standard deviation of the mean (Rodríguez-Miranda et al., 2014).

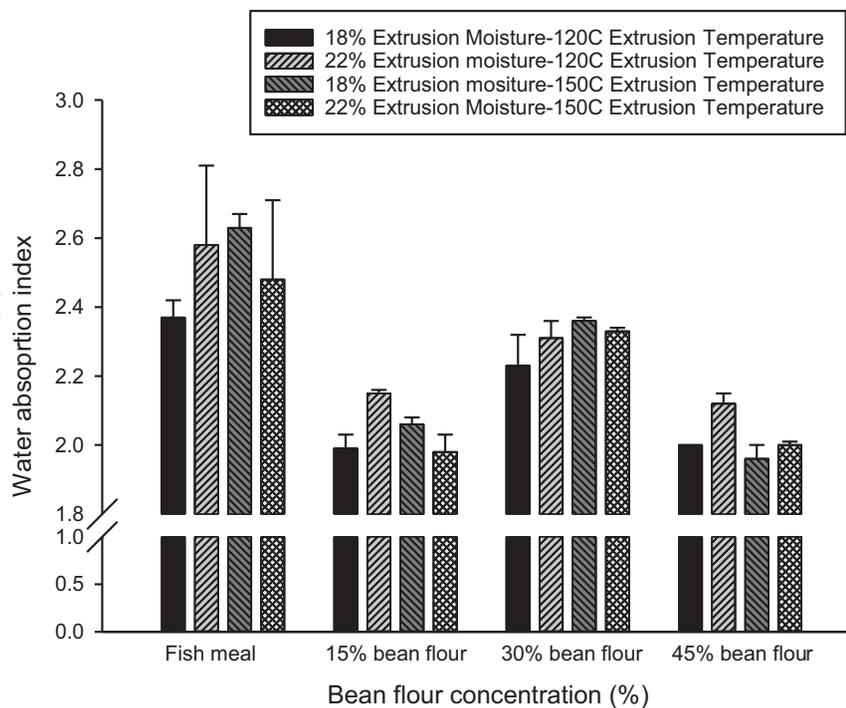


Figure 4. Water Absorption Index of extruded aquaculture feed at different extrusion moisture with fishmeal and different concentrations of bean flour (*P. vulgaris*). Vertical bars indicate standard deviation of the mean (Rodríguez-Miranda et al., 2014).

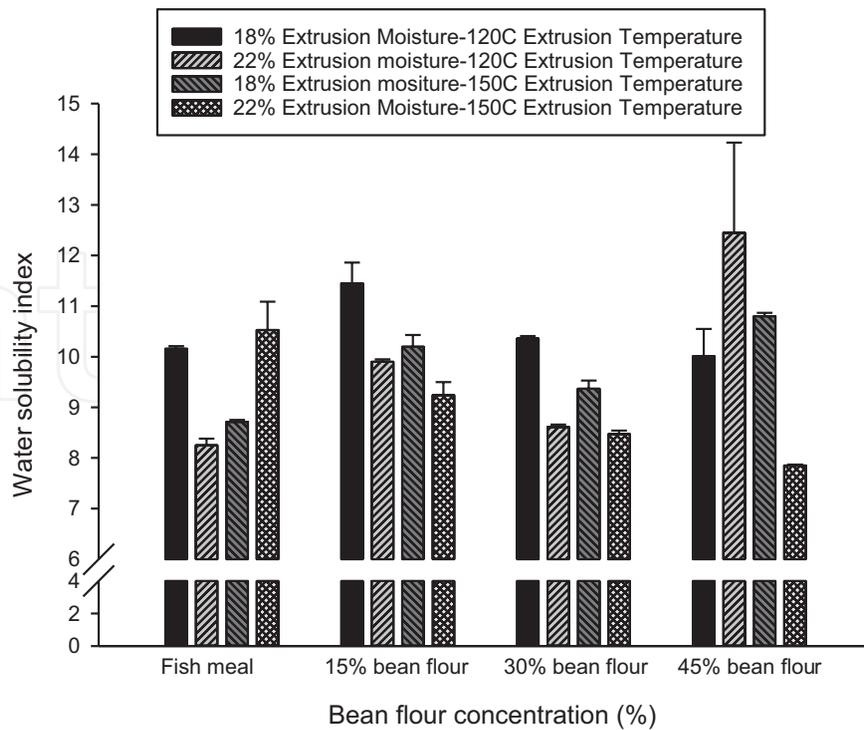


Figure 5. Water Solubility Index of extruded aquaculture feed at different extrusion moisture with fishmeal and different concentrations of bean flour (*P. vulgaris*). Vertical bars indicate standard deviation of the mean (Rodríguez-Miranda et al., 2014).

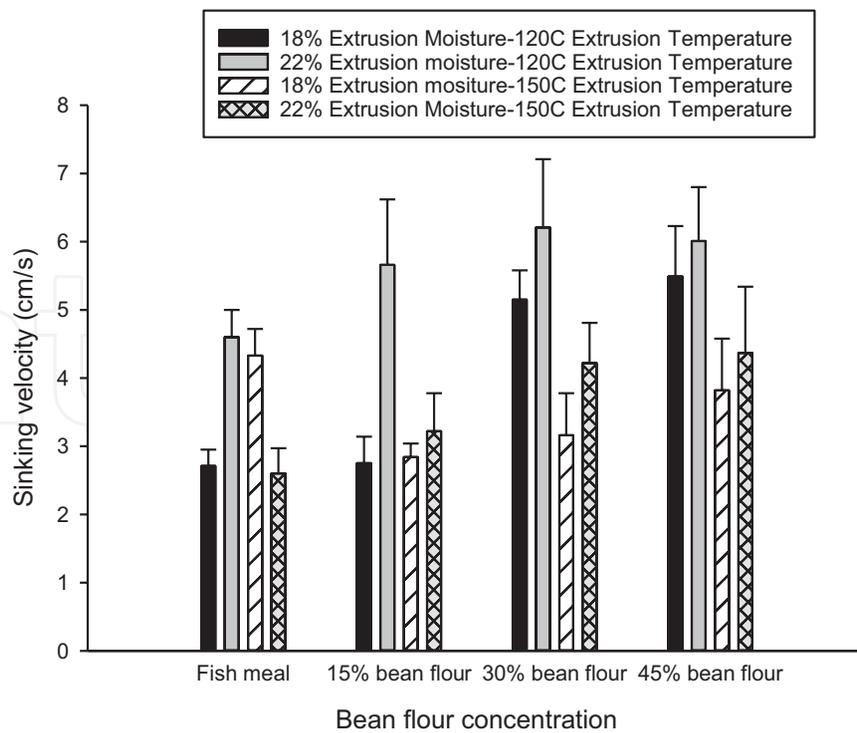


Figure 6. Sinking velocity of extruded aquaculture feed at different extrusion moisture with fishmeal and different concentrations of bean flour (*P. vulgaris*). Vertical bars indicate standard deviation of the mean (Rodríguez-Miranda et al., 2014).

the sinking velocity compared to extruded feed at 150°C. Bean flour can also affect ($p < 0.05$) the sinking velocity of the extruded feed. When feed contains bean flour and is required to have a low sinking velocity, the most recommended extrusion temperature is 150°C.

The independent variables to be considered in aquaculture extrusion are temperature, moisture content, and screw speed. Based on the independent variables and the dependent variables (Expansion Index, bulk density, and sinking velocity), in which the EI should be between 0.88 and 1.11, the bulk density ranges between 0.55 and 0.97 g/cm³ and the sinking velocity is required to be between 2 and 6.2 cm/s. The best extrusion conditions with a single laboratory extruder (Brabender, Germany) are 120°C, 22% moisture content at a screw speed of 140 rpm with a diet formulation containing 62% fishmeal and no vegetable protein. Diets containing 15 and 30% bean flour require less moisture (18%), but the same temperature and screw speed, to obtain an optimum aquaculture feed. Diets containing 45% bean flour and 15% soy protein are best extruded at 120°C and 18% moisture at a lower screw speed (80 rpm). The lower mineral content appears to affect in this case the screw speed. A sharp increase in soy protein concentrate of 30 and 45% requires high extrusion temperatures of 135 and 150°C, respectively. Extrusion of 30% soy protein requires 20% moisture content and a screw speed of 110 rpm. The moisture requirements are also high (22%) as well as the screw speed (140 rpm) for extrudates with 45% soy protein concentrates.

The specific mechanical energy (SME) is the necessary energy in the form of work in the extrusion process. In aquaculture feed, the SME is affected ($p < 0.05$) by the extrusion temperature. High extrusion temperature decreases ($p < 0.05$) the SME. The extrusion moisture does not affect ($p > 0.05$) the SME, except with samples containing 15% bean flour. When extruding samples containing bean flour, the increase in screw speed will increase ($p < 0.05$) the SME, but not with those materials containing fish meal and no bean flour [19].

5. Starch pregelatinization

Pregelatinized and not pregelatinized starch has been added to balanced aquaculture feed to study the effect of pregelatinization on the functional properties of the extrudates. The studies have shown that pregelatinization of starch before extrusion has a positive effect ($p < 0.05$) on the Water Solubility Index (WSI) (data not shown). The WSI decreases and can be beneficial for the aquaculture industry. Pregelatinization does not affect ($p > 0.05$) the Water Absorption Index of the extruded feed, while the sinking velocity of the extrudates does not increase ($p > 0.05$) compared to the diets with no starch. Adding starch to the extrudates will lower ($p < 0.05$) the sinking velocity of the extrudates, as long as the starch is not pregelatinized. Pregelatinized extrudates are harder than extrudates containing starch that have not been pregelatinized before extrusion. The Expansion Index (EI) of extrudates containing pregelatinized starch decreased ($p < 0.05$) compared to extrudates with starch which was not gelatinized. The decrease in EI was observed with 20% of starch content, but there were no differences ($p > 0.05$) with extrudates containing more than 50% starch [20].

Nixtamalization can also be used to pregelatinize starch. Nixtamalization is a traditional thermal treatment used for corn products in North and Central America, where corn kernels

are cooked with CaOH, resulting in a pregelatinized dough suitable for extrusion. **Figure 7b** shows pregelatinized corn kernels, where the center of the kernels appears to be enzymatically degraded during the steeping time of the process. **Figure 7a** shows a bean starch kernel before extrusion, while **Figure 7c** shows the structural matrix of extruded bean/corn flours. Again we observe the protein structure, but also partial gelatinization of the starch kernels. Different raw material influences the final product characteristics. Crystallinity represents the structure arrangement and is mostly related to the starch structure in the kernel. Although corn has a higher starch content (about 65.5%) [8], than bean flour (51.9%), bean flour shows a higher crystallinity than nixtamal (**Figure 8**). Extrusion decreases crystallinity because of the gelatinization of the starch kernels, but the temperature and moisture content during extrusion also affect the percentage of the crystallinity of the extruded product. The crystallinity of the extruded product is related to the retrogradation of the starch. Low extrusion temperatures produce the lowest crystallinity of the end-product probably because of the lower degree of gelatinization. High extrusion temperatures yield a low crystallinity because of starch dextrinization during extrusion and lower retrogradation.

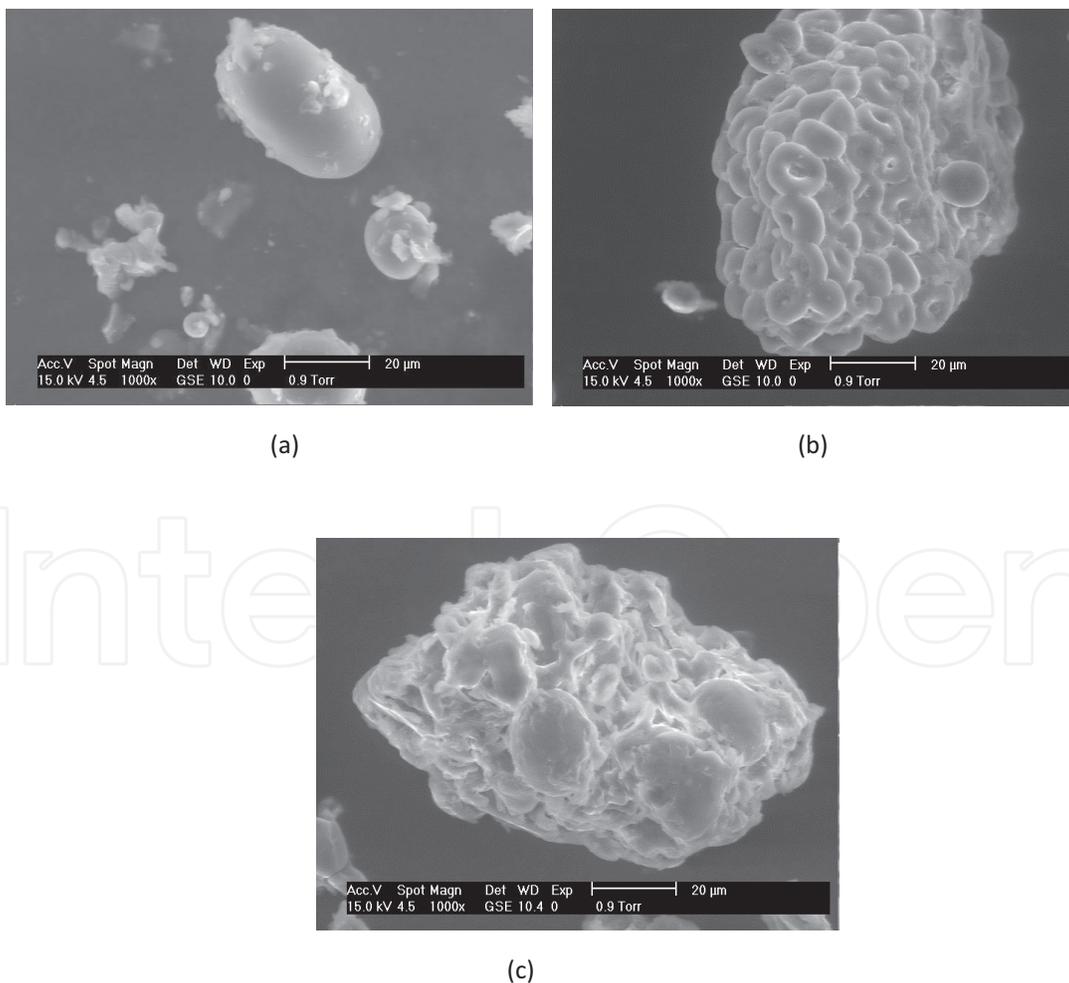


Figure 7. Scanning electron micrographs (1000 × magnification) of (a) bean flour, (b) nixtamalized corn flour, and (c) extruded bean-nixtamalized corn flour at 150°C and 14% H₂O (bar = 20 µm) (reproduced with permission from Atienzo-Lazos et al. [7]).

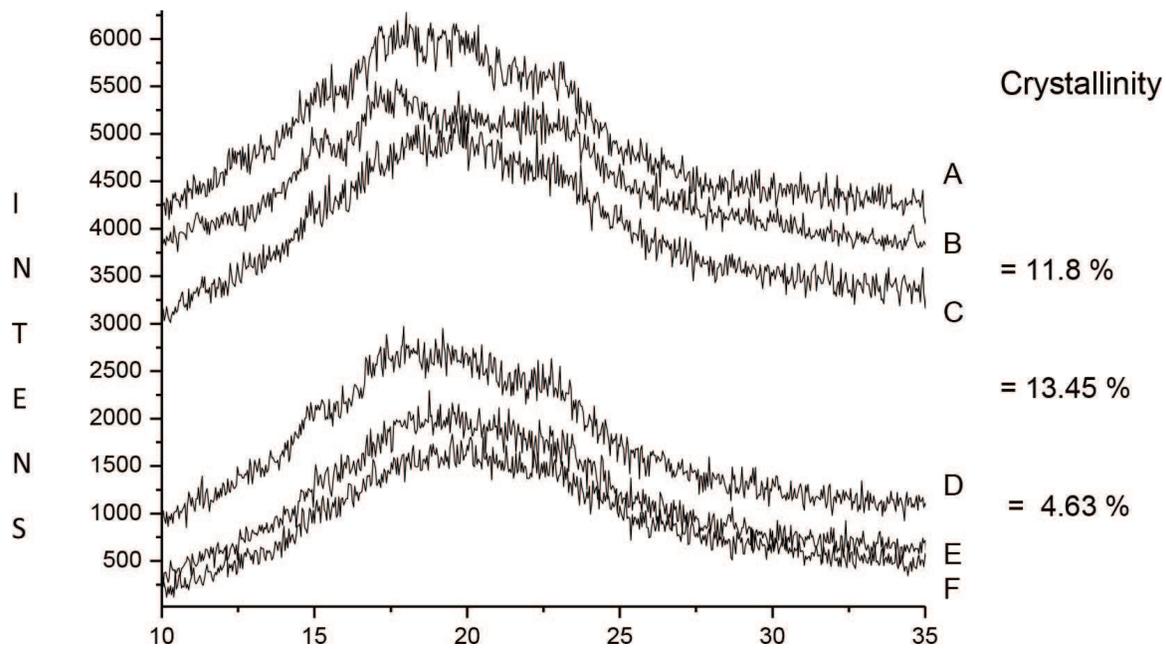


Figure 8. X-ray diffraction of A = Nixtamal flour, B = Bean flour, C = extruded flour at 150°C and 14.5% moisture, D = extruded flour at 170°C and 16.3 % moisture, E = extruded flour at 170°C and 18.7 % moisture, F = extruded flour at 190°C and 14.5% moisture (reproduced with permission from Atienzo-Lazos et al. [7]).

6. Feeding trials of extruded aquaculture feed

Studies of extruded trout feed show a final weight decrease ($p < 0.05$) after 32 days of feeding rainbow trout (*Onchorhynchus mykiss*) with bean flour. **Figure 9** shows a decrease ($p < 0.05$) in trout final weight and weight gained after being fed with extruded feed containing 15–45% of bean flour when compared to extruded feed-containing fishmeal. Trout fed with 45% of bean flour only gained 5.5% of weight in 32 days. Although weight gain is lower ($p < 0.05$) when fishmeal is substituted with bean flour, the survival rate was 100% for all the diets [21], indicating that bean flour can be used up to 30% as a fishmeal substitute. The feed conversion efficiency can be calculated as shown in Formula 1. The feed conversion efficiency (FCE) is highest ($p < 0.05$) with fishmeal (54.4%), and decreased ($p < 0.05$) to between 47.1 and 46.5% with 15 and 30% of bean flour, respectively. Extruded feed containing 15–30% bean flour shows an acceptable feed conversion efficiency. Feeds containing 45% of bean flour are not recommended for trout feed due to the low FCE Index.

$$\text{FCE} = (\text{Final weight(g)} - \text{Initial weight(g)}) / \text{Cconsumed feed(g)} * 100 \quad (1)$$

The condition factor or coefficient of condition K is a quality parameter of the fish and takes into consideration the weight and length. A condition factor above 1.6 shows that a fish has an excellent condition. The diets containing fishmeal and bean flour (15 and 30%) had a similar K factor (**Figure 10**). The K factor increased ($p < 0.05$) with 45% of bean flour. The diets containing fishmeal or 15 and 30% of bean flour had the same ($p > 0.05$) feed conversion ratio (FCR). The feed conversion ratio shows the inverse relationship between feed intake and weight gain of the fish and is related to the digestibility and metabolic use of the diet [22]. Trout fed with fishmeal or 15 and 30% of bean flour also had the same ($p > 0.05$) specific

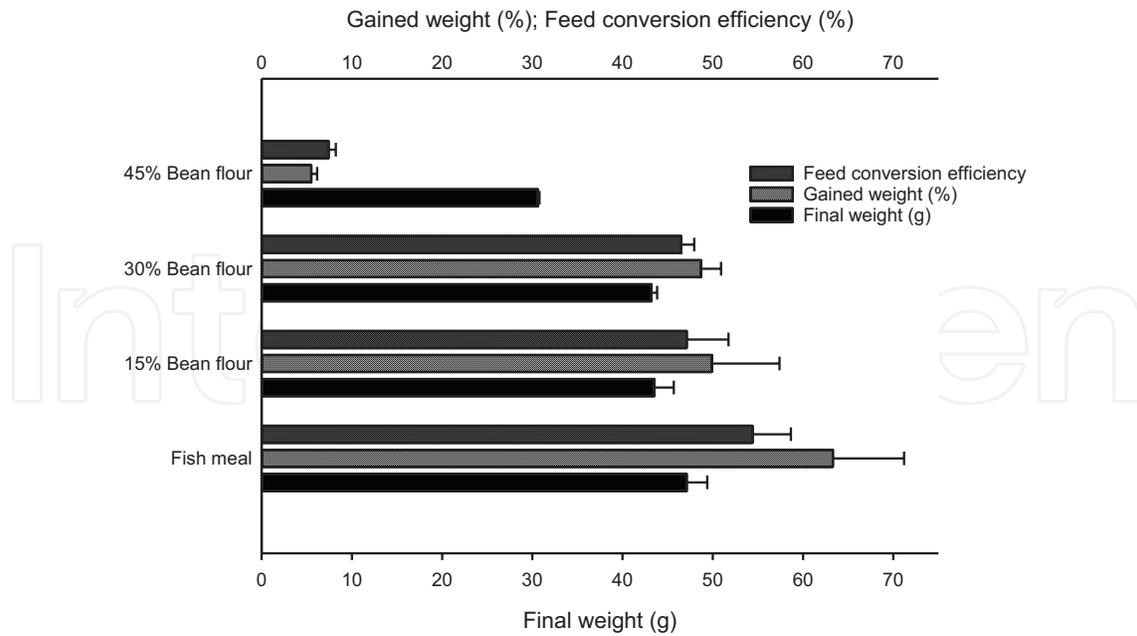


Figure 9. Feed conversion efficiency (FCE), gained weight, and final weight of rainbow trout (*O. mykiss*) fed with extruded aquaculture feed with fish meal or different concentrations of bean flour (*P. vulgaris*) for 32 days. Initial average weight = 29.1 g. Horizontal bars indicate standard deviation of the mean (adapted from Rodríguez-Miranda et al. [21]).

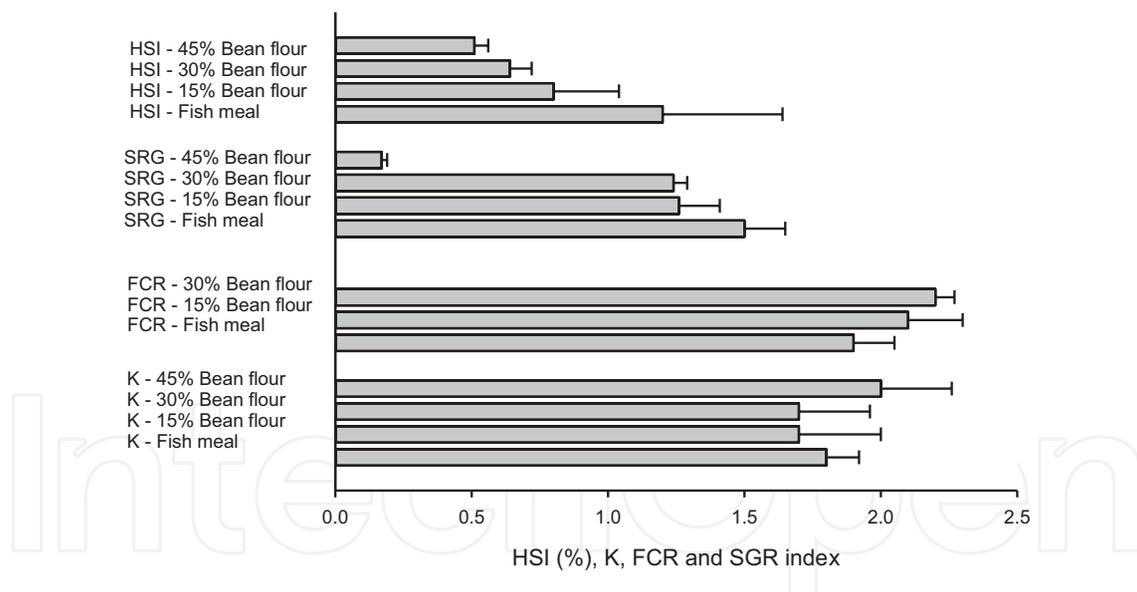


Figure 10. Condition factor (K), feed conversion ratio (FCR), Feed Conversion Efficiency (FCE) Index, and Hepatosomatic Index (HSI) of rainbow trout (*O. mykiss*) fed with extruded aquaculture feed with fish meal or different concentrations of bean flour (*P. vulgaris*) for 32 days. Initial average weight = 29.1 g. Horizontal bars indicate standard deviation of the mean (adapted from Rodríguez-Miranda et al. [21]).

growth rate (SGR), but not trout fed with 45% of bean flour in their diets. The Hepatosomatic Index (HSI) shows an indirect relationship between liver weight and body weight; it indicates the nutritional state of the trout. High HSI indicates a better fish nutritional condition. The fishmeal and the 15% bean flour diets have the highest HSI; there is no difference ($p > 0.05$) between the two diets. The diets with 30 and 45% of bean flour have lower ($p < 0.05$) HSI than the diets containing no fishmeal, and either no bean flour or 15% bean flour.

7. Color of extruded bean flour aquaculture feed

The L^* values describe the lightness of the color of the sample on a scale of 0–100, where 0 = black and 100 = white. On the other hand, the a^* values if positive (0–60) are related to a reddish color of the extruded product, if the a^* values are negative (0–60), the feed tends to be greener. The closer the values are to zero the more the color tends to be neutral. The values with the highest ($p < 0.05$) luminosity have the lowest a^* values. The samples with higher ($p < 0.05$) L^* correspond to the extrudates with 45% of bean flour, probably because of the presence of more starch in the sample. These samples also have the lowest ($p < 0.05$) a^* . The samples with the lowest L^* are the samples without bean flour, except the sample extruded with 15% bean flour, 150°C, and 22% moisture content, which also has a low L^* . The samples with less bean flour and more fishmeal tend to have a higher a^* value (**Figure 11**). The major factor affecting

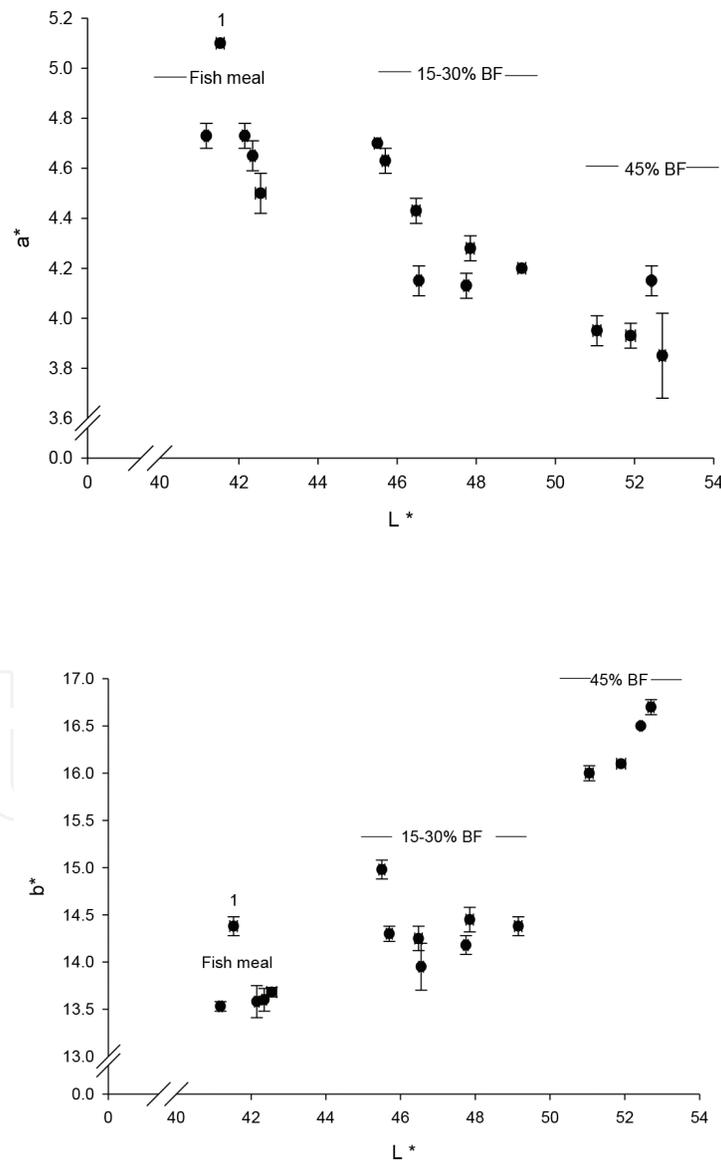


Figure 11. Lightness (L) in function of a^* and b^* of extruded aquaculture feed at different extrusion moisture with fishmeal and different concentrations of bean flour (*P. vulgaris*). BF = Bean flour; 1 = extruded at 150°C/22% moisture, and 15% BF; Vertical and horizontal bars indicate standard deviation of the mean (adapted from Rodríguez-Miranda et al., 2014).

L^* and a^* is the content of bean flour and fishmeal. Positive b^* values (0–60) represent a yellow color and negative values (0–60) are blue. The samples with fishmeal and no bean flour (BF) have values ranging between 13.53 and 13.68 lower ($p < 0.05$) than the b^* values for the extruded samples with bean flour, except the sample with 15% BF extruded at 150°C and 22% moisture. The extruded aquaculture feed with 15–30% of bean flour present a less gray color tending to yellowish with values between 14.18 and 14.98. For higher amounts of bean flour, the feed tends to be lighter in color and more yellowish (16.00–16.7). The differences in color are explained in part by the original color of the raw material; fishmeal tends to be more into the gray, less light color, while starch present in the bean flour also affects luminosity and color. The thermal process during extrusion also defines the final color of the feed. Different chemical reaction, such as milliard and caramelization interact to give the final luminosity and color of the product.

8. Effect of extrusion on bioactive compounds

The effect of extrusion moisture and temperature on the antioxidative capacity and bioactive compounds in bean/corn extrudates is shown in **Table 4**. Neither extrusion temperature nor extrusion moisture had an effect ($p > 0.05$) on the antioxidant activity [23]. The β -carotene, flavonoids, or polyphenols content was not affected ($p > 0.05$) by the extrusion temperature and moisture. An experimental central rotary design of second order was used for the extrusion experiment. The experiments were conducted in a single screw extruder with a temperature range from 142 to 198°C and 16.3 to 18.7% moisture content. Extrusion even at 192°C does not seem to affect the antioxidative activity, nor the concentration of the active compounds. Extrusion uses little processing time, which makes it an adequate way for food processing since it is not shedding for bioactive compounds.

| | Intercept | Lineal | | Quadratic | | Interaction | R ² |
|---|-----------|--------|--------|-----------|--------|-------------|----------------|
| | b_0 | X_1 | X_2 | X12 | X22 | X_1X_2 | |
| CUPRAC (μM Trolox equivalent/g) | -368.566 | 1.089 | 37.097 | -2.83E-3 | -1.060 | -0.011 | 0.116 |
| β -carotene (%) | -382.798 | 2.403 | 22.795 | -3.79E-3 | -0.324 | -0.066 | 0.112 |
| Flavonoids (mg Catechin equivalent/g) | -288.451 | 1.754 | 17.586 | 2.91E-3 | -0.266 | -0.049 | 0.157 |
| Polyphenols (mg Gallic acid equivalent/g) | -184.111 | 1.133 | 11.783 | -2.07E-3 | -0.209 | -0.027 | 0.114 |

*Significant difference ($p < 0.05$). X_1 = temperature [°C], X_2 = moisture content [%].

Table 4. Regression coefficients of the response surface model of extruded bean 60%-bean/40%-corn extrudates (Delgado-Licon et al. [23]).

9. Extruded cottonseed meal

The use of cottonseed meal (CSM) in extruded snacks can double the amount of protein with just an increase of 10% CSM [9]. The protein concentration of an extruded snack can enhance

from 6.4 to 12.8% when 10% CSM was added to the formulation. **Table 1** shows the protein content of different agricultural by-products and their chemical composition.

The difference in chemical composition changes the chemical and physical structure, the extrusion properties, and the functional properties of the final product. **Figure 12** shows two extruded samples. **Figure 12a** illustrates the structure of extruded corn masa, with a low protein content and a high starch content. It can be seen how layers of starch are built to provide expansion to the extruded product. On the other hand, **Figure 12b** shows the matrix of extruded cottonseed meal, which has 12.8% of protein. The flat, homogenous layers are gone, and a more irregular structure is present. It appears as if the protein breaks up the continuous starch structure and builds a less homogenous texture. The lower concentration of starch and the presence of more protein produce a more compact structure, which has a Lower Expansion Index and a harder crispier structure. The increase of protein content in extruded corn/cottonseed meal products reduces ($p < 0.05$) the physical and functional properties of the end-product; the Expansion Index, the water activity, and the water absorption and solubility indices decrease ($p < 0.05$) with protein cotton increase. As Expansion Index decreases the hardness of the extruded products increases. It is not only the low starch concentration that lowers the Expansion Index of the extruded products, but the matrix composition and structure also determines the hardness and Expansion Index of the final product. **Figure 12** shows how proteins produce a more compact irregular structure in cottonseed meal (CSM) extruded products. When CSM is extruded in a single screw extruder, an increase in CSM negatively affects ($p < 0.05$) the Expansion Index, because of the presence of protein. CSM also decreases ($p < 0.05$) the water activity, Water Absorption Index, and the Water Solubility Index of the extruded product [9]. In aquaculture, Low Water Solubility and Low Water Absorption Indices are most likely to be preferred, rather than high values. The extruded feed requires stability in an aqueous environment to assure that the fish or shrimp can have time to consume it. Stability of the extrudates also helps to reduce water turbidity and pollution. Low Water Solubility and Water Absorption Indices have a positive effect on the quality of aquaculture feed. On the other hand, a lower water activity (a_w) is related to a longer shelf life of the feed.

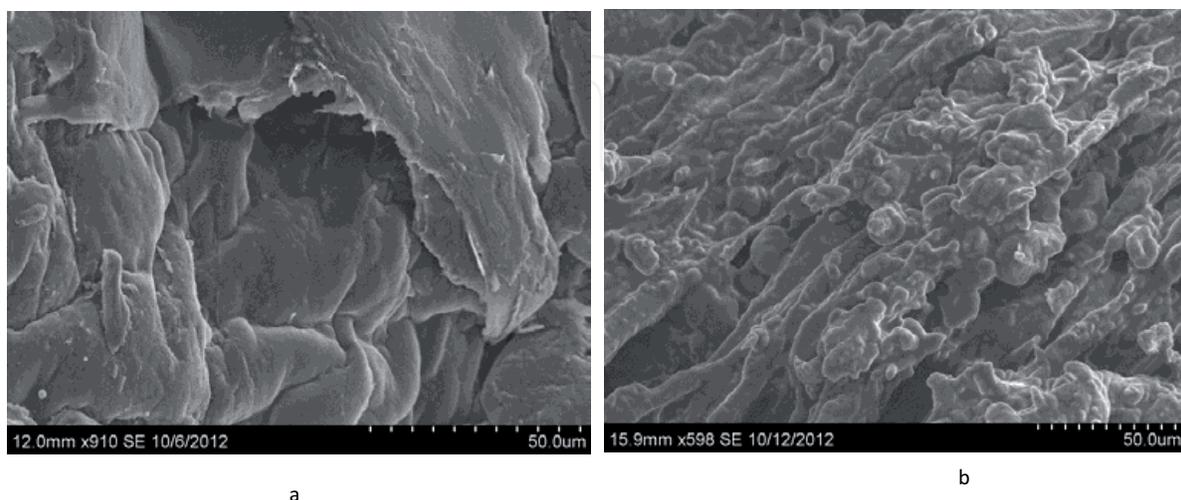


Figure 12. Scan microscopy of extruded (a) corn masa and (b) cottonseed meal (reprinted with permission from Reyes-Jaquez et al. [9]).

Extrusion shows restructuring of cottonseed meal. **Figure 13a** shows a heterogeneous structure before extrusion and a homogenous structure after extrusion (**Figure 13b**). Lambda scan microscopy of extruded products shows different scans between samples with and without cottonseed meal (**Figure 14**). The extruded samples with cottonseed meal show a second pick at about 670 nm (**Figure 14a**), which is not shown in the samples without cottonseed meal (**Figure 14a**).

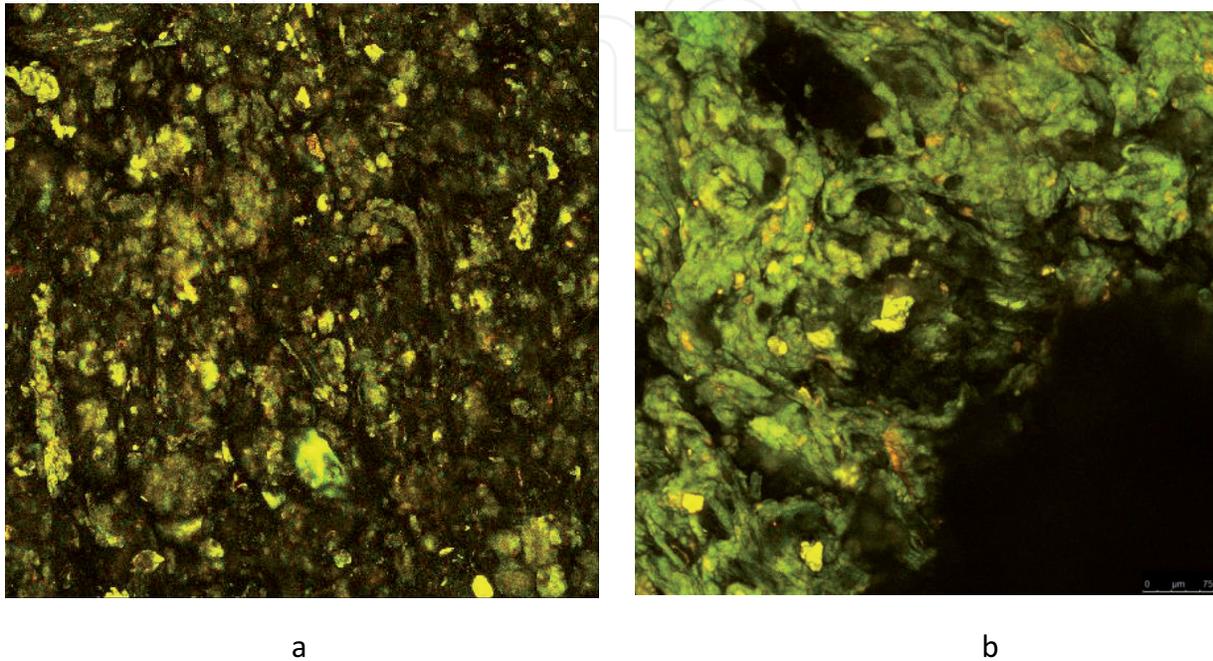


Figure 13. Confocal microscopy of (a) not extruded and (b) extruded cottonseed meal (reprinted with permission from Reyes-Jaquez et al. [9]).

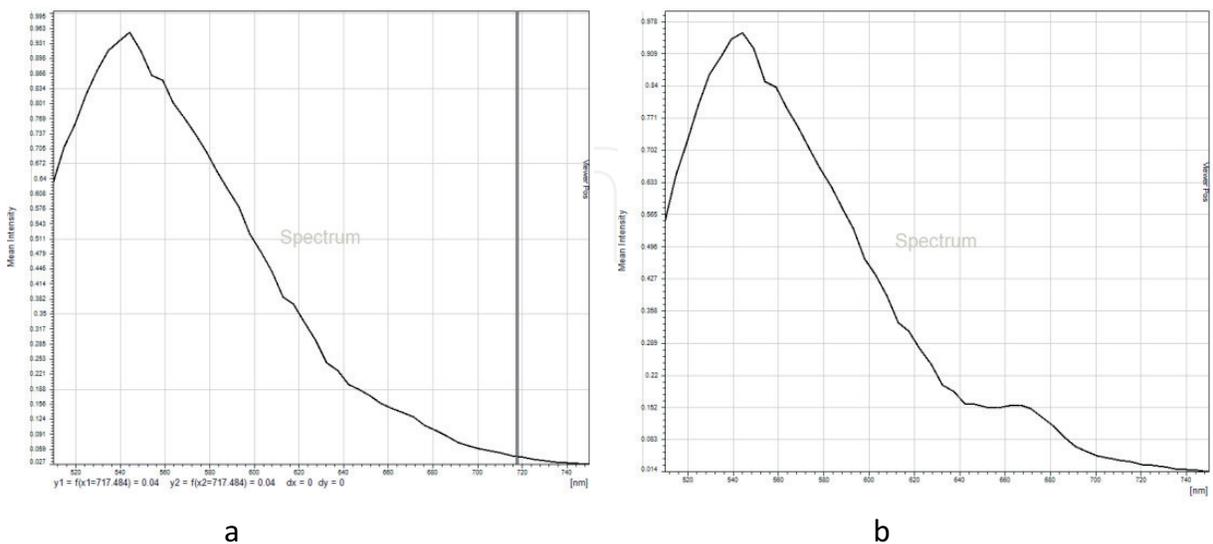


Figure 14. Lambda scan microscopy of extruded (a) corn masa and (b) corn masa/cottonseed meal (reprinted with permission from Reyes-Jaquez et al. [9]).

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