Preconditioner influence on twin-screw extrusion cooking of starch-based feed pellets: The example of Fish Feed

Asma Chaabani, Laurent Labonne, Vanessa Durrieu, Antoine Rouilly, Fabien Skiba, Philippe Evon

To cite this version:

Asma Chaabani, Laurent Labonne, Vanessa Durrieu, Antoine Rouilly, Fabien Skiba, et al.. Preconditioner influence on twin-screw extrusion cooking of starch-based feed pellets: The example of Fish Feed. Aquacultural Engineering, 2022, 98, pp.102268. 10.1016/j.aquaeng.2022.102268 . hal-03702896

HAL Id: hal-03702896
https://hal.inrae.fr/hal-03702896v1

Submitted on 22 Jul 2024

Preconditioner influence on twin-screw extrusion cooking of starch-based feed pellets: the example of *Fish Feed*

Asma Chaabani [a,b], Laurent Labonne [a], Vanessa Durrieu [a], Antoine Rouilly [a], Fabien Skiba [b], Philippe Evon [a,*]

[a] Laboratoire de Chimie Agro-Industrielle, LCA, Université de Toulouse, INRAE, Toulouse, France

[b] Nutricia, Haut-Mauco, France

* Corresponding author.

E-mail address: Philippe.Evon@toulouse-inp.fr (Ph. Evon).

Abstract

This work compares the performance of pellets for adult Trout obtained in two different processing conditions. The first one consists of adopting the traditional *Fish Feed* process based on a preconditioning steam treatment step before twin-screw extrusion. The second one was a simplified process based only on a twin-extruder ensuring both the cooking and the extrusion of fish pellets in a single step. Six formulations were obtained while–maintaining feed specifications (36 % of proteins and 30 % of lipids). The comparison between the two processing methodologies (*i.e.*, with or without preconditioning) was then based on two fundamental aspects. Firstly, the gelatinization rate of starch contained inside pellets was determined. Secondly, the pellet's characteristics (bulk density, expansion ratio, diameter and pellet's porosity) and their usage properties (oil leakage rate, durability, hardness and floatability) were evaluated. The present work has evidenced that it is possible to produce pellets without preconditioning while ensuring a total starch gelatinization and maintaining their usage properties. The *Fish Feed* process has thus been partially simplified.

Keywords

- *Fish Feed*
- Twin-screw extrusion process
- Preconditioning
- Moisture
- Expansion
- Starch gelatinization
- Physical properties

1. Introduction

Historically, *Fish Feed* has always been produced in a pelletized form. Using a simple technology such as a ring die pellet mill, pelletized feed is produced. It is rather stable and cheap in production cost. However, its main disadvantage is that there are limited possibilities to influence the physical properties of pellets, especially their shape and density **(Burel, 2017; Terpstra, 2015)**. Yet, the diameter of commercial extruded *Fish Feed* can range from 0.5 to 30 mm. In fact, the size of the pellet must adapt the fish size because this animal swallows the feed as an intact particle **(Terpstra, 2015).** Furthermore, thanks to the extrusion technology, it is possible to have a wide range of bulk densities (from 350 to 750 g/L). Both sinking and floating pellets can ensure satisfactory growth **(Craig, 2009)**. However, some farmers prefer floating feed, which can help them to directly observe the feeding intensity and therefore to adjust feeding rates **(Khater et al., 2014)**. The three main constituents inside pelletized feed are starch, proteins and lipids. If the last two ones are added for nutritional considerations, the main interest to add starch is to give cohesion to the pellet. However, starch is only partially cooked inside such pellets, making its digestion by fish more difficult. For the same reason, pellets become soft in the presence of water, thus contributing to oil leakage **(Terpstra, 2015)**. To overcome these limitations, extrusion has found its place in aquaculture for nearly fifty years, becoming nowadays the primarily used technique for aquafeeds (**Kraugerud and Svihus, 2011; Rokey, 1994; Sørensen, 2012**). Thanks to this continuous technology, extruded feeds are of better quality with a clear improvement in some physical characteristics, e.g., a firm structure, the absence of dust, adjustable size and shape, and the stability in water **(Terpstra, 2015)**. . Extrusion leads also to higher inclusion of lipids, and the density of the pellets is easily adjustable. This technique may be defined as a continuous cooking process in which a food or feed material is forced to flow, under elevated pressure, temperature and shear, through a die to give unique physical and chemical functionality to food materials **(Altan et al., 2009; Riaz and Rokey, 2011)**. During this thermomechanical process, different functions can be performed such as ingredients agglomeration, starch gelatinization, expansion, dehydration, mixing, homogenization, protein denaturation, pasteurization, shearing, texturizing, and product shaping **(Riaz and Rokey, 2011)**.

In *Fish Feed* manufacturing, the technological process applied nowadays involves the following operations: raw materials preparation (weighing, mixing, grinding and sieving), preconditioning, extrusion, drying, fat coating, cooling, sieving and packaging. An extrusion system consists of a barrel situated between the preconditioner and the pelletizing knife. This key part of the extruder is made of one or two rotating screws for the cooking and raw materials processing. Extruders are classified in different ways, but the most common ones are based on the screw design (single- or twin-screw extruders), the source of heat and the level of moisture in the material during the process (dry and wet extruders) **(Riaz and Rokey, 2011)**. In *Fish Feed*, most of the extruders used are single-screw ones. However, and even if the twin-screw machines are much more expensive, these are known for their versatility **(Riaz and Rokey, 2011)**.

Positioned between the dry mix feeder and the extruder barrel, the preconditioner is generally used in animal feed extrusion processing (*e.g.*, aquafeeds), especially when the moisture content exceeds 18 % **(Bortone, 2017; Riaz and Rokey, 2011)**. During preconditioning, the raw material particles are mixed homogeneously and continuously with liquid water and steam for a given time before extrusion, to hydrate and heat the particles **(Riaz and Rokey, 2011)**. Hydrothermal cooking of the raw material starts during this step, resulting in the swelling of flour particles as well as in the pre-gelatinization of starch granules. At this level, particles are heated to a temperature of 80 °C to 95 °C **(Rokey, 1994)**, and hydrated to a moisture content ranging between 18 % and 30 % (w/w) **(Riaz and Rokey, 2011; Strahm, 2000)**. As pre-heating is much faster than pre-humidifying, the preconditioning step is thus firstly controlled by the transfer of moisture into the particles **(Lavocat and Raymond, 2018)**. On the one hand, the hydration time is longer with a bigger size of the particles. On the other hand, it can be reduced thanks to a warmer environment. An efficient moistening and swelling of the particles can only be achieved thanks to a suitable residence time of the raw material in the preconditioner. In other terms, the preconditioner function is to provide the extruder with an evenly moistened and preheated mix. The quality of the preconditioning stage thus depends on the efficiency of heating, hydration, and mixing **(Riaz and Rokey, 2011)**.

The first developed equipment was a single-shaft preconditioner. The latter ran at high speed to attain a good mixing but had a reduced retention time of only 30 s or less, which is now considered ineffective by today’s standards **(Riaz, 2000)**. This is why a double-shafted preconditioner was developed, and almost dominates nowadays the pellet feed industry **(Pandey, 2018)**. Such preconditioner is made of a tank in which counter-rotating twin-shafts equipped with paddles are located. Water is injected with two nozzles located on both sides of the tank entrance. Steam is generally injected through two to six points on the first half of the tank. The spray nozzles help to distribute water homogeneously on the raw particles and thus reduce the required mixing intensity **(Riaz, 2000)**. The performance of the preconditioning step depends on several operating conditions, *e.g.*, the number and orientation of the paddles, the steam, and water flow rates in relation with the raw material one, and the rotation speed of the twin-shafts **(Riaz and Rokey 2011)**. All these conditions have an impact on the efficiency of the heating and pre-humidifying process, as they influence the residence time of the raw material inside the preconditioner, its filling ratio, its mixing ability, and lastly the energy and mass transfers. Depending on the paddle configuration (mounted with direct or reverse pitch), the average filling ratio can be modulated and, consequently, the mean residence time of the mixture inside the preconditioner **(Riaz and Rokey, 2011)**. The filling ratio is defined as the volume occupied by the raw material in relation to the total volume of the preconditioner. Increasing this parameter improves the hydration and temperature uniformity of the mix **(Bortone, 2017)**. In addition, depending on the flow rate of steam injected, the starch gelatinization rate varies: the more steam injected, the more starch gelatinization occurs **(Lavocat and Raymond, 2018)**. A reduced flow rate of the raw material also tends to increase its mean residence time in the preconditioner, thus resulting in improved gelatinization of starch **(Lavocat and Raymond, 2018)**. Lastly, a reduced rotation speed of the twin-shafts increases the mean residence time in the preconditioner. However, a slight decrease in the gelatinization rate of starch may be evidenced in such a situation **(Lavocat and Raymond, 2018)**.

In conclusion, the preconditioner **(Fig. 1)** is considered today as vital equipment in *Fish Feed* manufacturing. Its performance indeed directly influence the starch pre-gelatinization rate, which can reach 60-70 % at the preconditioner outlet, or even more (up to around 90 %) when working with a particularly low inlet flow rate of solid material **(Lavocat and Raymond, 2018)**. **Riaz and Rokey (2011)** had reported that to improve the extrusion-cooking step and the final product quality, at least one-third of starch gelatinization must occur in the preconditioner. The latter contributes to an efficient heat transfer through friction inside the extruder barrel, just as a reduction in its energy consumption **(Sørensen, 2012)**. It also lengthens the life of the extruder by reducing the abrasive wear of the barrel and screws **(Sørensen, 2012)**. Lastly, the preconditioner is also interesting for sanitary purposes as this heating step can help to control or reduce salmonella levels in the raw materials **(Riaz and Rokey, 2011)**. At an industrial scale, using a preconditioner can also lead to increasing the extruder productivity, and to an improvement in the final product quality, *e.g.* its water stability **(Bortone, 2017)**, density, and hardness. Recently, new preconditioner designs have even been developed for new ranges of aquafeed **(Riaz, 2019)**.

Next to the preconditioner, the conditioned feed mixture is thus transferred to the extruder to be transported, melted, cooked, heated, and mixed at high temperature (120-130°C) and high pressure (20-30 bar) **(Sørensen, 2012; Terpstra, 2015)**, and finally shaped through the die. This device contains as many holes as required. The die determines the extruded pellet's shape and size **(Riaz and Rokey 2011; Terpstra, 2015)**. Finally, pellets are cut to the desired length, thanks to a rotating knife positioned immediately after the die. When the extrusion-cooked solid material leaves the die, an expansion phenomenon occurs through water evaporation. Small water droplets that were dispersed inside the solid pass from high to atmospheric pressure. Moisture vaporizes and expands pellets through the formation of pores **(Williams, 2000)**. These small pores are then filled by oil at the moment of the coating extra-step in the case of the high-energy diets **(Sørensen et al., 2010)**.

In the extrusion system, energy comes from two main sources, *i.e.*, mechanical, and thermal **(Sørensen, 2012)**. The first one is known as the specific mechanical energy (SME) which is the amount of mechanical energy dissipated as heat inside the material **(Altan et al., 2009)**. According to **Mercier et al (1989)**, SME is the product of torque and screw speed divided by mass flow rate. SME provides a good characterization of the extrusion process **(Altan et al., 2009)** and its value indicates the extent of molecular breakdown and eventually material degradation **(Godavarti and Karwe, 1997)**.

The extrusion technology has many advantages among which versatility and its ability to obtain a high-quality product **(Gaosong and Vasanthan, 2000; Riaz and Rokey, 2011)**. Given the fact that extrusion is a high-temperature short time (HTST) heating process, degradation of food nutrients is minimized, and the digestibility of dietary components is improved **(Riaz and Rokey, 2011)**. In addition, it contributes to destroy the anti-nutritional compounds, some undesirable enzymes and microorganisms **(Riaz and Rokey, 2011)**, just as it inactivates heat resistant toxins (*e.g.* aflatoxin) **(Saalia and Philips, 2011)**. When extrusion conditions are not well optimized, it can nevertheless reduce the availability of some nutrients, generate the cross-linking of lysine, and also destroy some vitamins and other thermolabile substances **(Rokey, 1994)**.

For salmonids, it is mainly dietary proteins and lipids that allow them to cover their energy needs. These animals have limited capacity to efficiently use and process dietary carbohydrates **(Médale and Guillaume, 1999)**. This results in feed rich in proteins and lipids, and with low content in carbohydrates **(Burel, 2017; Terpstra, 2015)**. Pellets with high-fat content are necessary to satisfy their nutritional requirements. However, during extrusion, the amount of fat that can be added is limited as it behaves as a lubricant, thus leading to a loss of mechanical shear **(Pandey, 2018)**. To overcome this disadvantage of extrusion, oil is commonly added through a coating extrastep. Firstly, the fat coating was practiced with atmospheric systems like drum coaters, mist-coating units, and paddle mixers **(Bortone, 2006)**. However, for a special feed diet like high-energy feed for salmons, oil coating can only be successfully achieved with vacuum coaters. Under vacuum, the air inside the feed pores is first removed, and coating then allows the injected oil to fill these pores when the coater's pressure get back to the atmospheric pressure **(Lamichhane et al., 2015)**. Dried pellets are transferred to the coater, which is subsequently placed under a vacuum. Then, oil is sprayed onto the extruded pellets and when the vacuum is released, the oil is forced into the pellet pores. Vacuum coating is an essential step to produce high-energy feed. It also enables the development of more nutritious feed containing functional additives **(Li et al., 2003)** like vitamins and exogenous enzymes that could not be added at the time of extrusion due to their thermolabile character **(Maas et al., 2020)**. Thus, thanks to the extrusion and expansion process that allows the creation of enough pore spaces, it is now possible to infuse a high level of fat (*i.e.*, up to 40 %) during the vacuum coating step. This is especially required for salmon feed **(Bell and Koppe, 2010; Dethlefsen, 2017)**.

The extrusion process allows *Fish Feed* producers to finely control the pellet density during production, the latter being easily adjusted to the eating habits of the farmed fishes (*i.e.*, sinking, semi-floating or floating pellets). Also, extrusion ensures greater feed stability in water with better production efficiency **(Clayton, 2002; Rokey, 2006)**. In fish farming, in particular intensive aquaculture, the physical properties of feed are in top priority. A particularly important property is the ability of feed to be handled from production to the fishpond without creating an excessive amount of dust **(Sørensen, 2012)**. Pellets need to maintain their integrity *via* high durability to minimize product loss during transport and pneumatic feeding. Thanks to its high versatility, the extrusion makes it possible.

The present work aims to develop an innovative twin-screw extrusion process to produce *Fish Feed* pellets for adult Trout. A twin-screw extruder is in fact capable of ensuring a wide range of functions like starch cooking, which is generally obtained partially in the preconditioner. In this context, the objective of this study is to highlight that the extruder alone could allow for the future the realization of the whole *Fish Feed* process. This study mainly focuses on the comparison of extruded pellets obtained with and without a preconditioner. The first section concerns essentially the starch gelatinization phenomenon, which is of key importance in *Fish Feed* processes. In the second section, a comparison of the obtained pellets in terms of their usage properties (durability, hardness, floatability and oil leakage rate) has been conducted to understand specifically the role of the preconditioner and the challenges to be raised in its absence.

2. Materials and Methods

2.1.Materials

Various ingredients were used to produce the extruded pellets. Some of them were used for their protein intake, whether of animal (fish meal) or vegetable origin (*e.g.*, soy protein concentrate, etc.). Others were cereal's (*e.g.*, wheat, corn) products and by-products. Nutricia (France) provided all these ingredients.

Extruded pellets were coated using three commercial oils, that were provided as well by Nutricia: two fish oils (F1 and F2), both obtained by wet pressing (0.92 g/cm^3 at 20 °C), and a crude rapeseed oil (R) obtained by cold mechanical pressing (0.91 g/cm^3 at 20 °C). To avoid their oxidation, F1 and F2 were stored in a dry place away from light before their use during the coating step.

Extruded pellets were composed of 12.2% (w/w) corn gluten, 12.6% fish meal, 23.0% soybean meal, 3.7% soy protein concentrate, 1.3% wheat gluten, 17.2% shelled faba bean, 3.0% wheat flour, 5.8% F1, 1.0% F2 and 20.2% R. Their chemical composition is summarized in **Table 1**. This composition was determined based on the chemical composition of each ingredient, and it has been developed to meet the nutritional requirements of adult Trout. In particular, the starch level was 11.1% (w/w) in proportion to the fresh matter (**Table 1**), thus ensuring both expansion at the extruder die outlet, and cohesion for the final pellets.

2.2.Feed recipe formulation

To prepare the recipe, ingredients were finely ground by a Hosokawa Micron (Summit, New Jersey, USA) ZPS Zirkoplex industrial air classifier mill. After their reception, all the ingredients were weighed using an Ohaus (Parsippany-NJ, USA) CD-11 digital balance, and they were then introduced into an Electra (Poudenas, France) MH 400 horizontal mixer. This mixer is equipped with double hollow spirals with reverse pitch to improve the mixing. The mixing operation was carried out for 20 min by changing the direction of rotation every 5 min to obtain a homogeneous solid mixture. Six batches (each 120 kg) were prepared before extrusion, resulting in the production of six different formulations of extruded pellets named S1 to S6.

2.3. *Fish Feed* production

Extruded pellets were produced using a Clextral (Firminy, France) Evolum HT 53 pilot-scale co-rotating and co-penetrating (*i.e.*, interpenetrating) twin-screw extruder. The S1 and S2 formulations were obtained according to the classical *Fish Feed* process presented in **Fig. 2** whereas the S3 to S6 formulations were produced without the preconditioner.

The solid mixture was introduced at a 110 kg/h flow rate (Q_S) in a Clextral (Firminy, France) Evolum preconditioner with a capacity of 75 L for the pre-cooking step. This preconditioner was equipped with two counter-rotating shafts with variable speed and adjustable blades. At this level, liquid water (Q_{PREC} for the corresponding flow rate) and steam (Q_v for the corresponding flow rate) were added to partially cook and pre-humidify the initial dry solid mixture. To conduct this task, a Covemat (Trivier sur Moignans, France) GE 490 steam generator and a Clextral (Firminy, France) DKM Super KL CAMP piston pump were connected to the preconditioner. After a residence time of about 3-4 min, the pre-moistened and pre-heated mixture was fed into the Clextral Evolum HT 53 twin-screw extruder. This machine has been designed to treat solid materials continuously at high pressure (from 1 to 275 bars) and high temperature (from 20 °C to 300 °C).

Extrusion parameters were the same for the six formulations produced. Only screw speed was changed: 425 rpm for the S1, S3 and S5 formulations, and 575 rpm for the S2, S4 and S6 ones. The barrel was composed of nine modules with an internal diameter (D) equal to 53.15 mm, each 4D in length. Screws had an outer diameter of 52.45 mm. The temperature profile was fixed as follows: 25 °C for module 1, 80 °C for modules 2 and 3, and 120 °C for modules 4 to 9. The screw profile was kept the same, and it was especially composed of two successive pairs of reverse screw elements used here to apply an intense mixing and mechanical shearing to the solid mixture, thus resulting in a significant increase of its residence time. After its conveying from the module 1, the solid mixture was mixed with an additional amount of water (Q_{TSE} for the corresponding flow rate), which was injected at the end of module 3 using a Clextral (Firminy, France) DKM Super KL-PP piston pump. Then, the reverse screw elements located at the end of module 7 were in fact the place where the solid mixture was compressed and plasticized, being converted into a dough-like state. Finally, the solid mixture was transported up to the die using conveying screws with decreasing pitch.

As the penultimate module was an open-top one, a Clextral (Firminy, France) vent-stuffer (*i.e.*, a vertical stuffing screw) was positioned on its top to maintain the extruded mixture inside the barrel during the extrusion process. A die made of one conical insert having a 5.9 mm outlet diameter was positioned at the end of the extruder barrel, and a Clextral (Firminy, France) HC 45 granulator knife was installed immediately after the die. By regulating its rotation speed, the continuous cutting of the extruded pellets to the desired length (*i.e.*, around 9.0 mm) was ensured.

For each formulation, the extrusion process was maintained for 10 to 15 min to reach a steady state before sampling the extruded pellets. Besides, samples were collected at the outlets of the preconditioner and the extruder to determine their moisture contents, and to carry out further characterizations. The process parameters (*i.e.*, the screw speed, motor current, barrel and material temperatures, and material pressure) were registered every 2 sec. Specific mechanical energy (SME) is directly related to the electrical power supplied by the motor (P), and it is expressed in W h/kg. In this case of the Clextral Evolum HT 53 twin-screw extruder used in this study, the operating voltage of the motor (U) is equal to 454 V, its theoretical efficiency (cos φ) is 0.90, and the maximum screw speed (S_{max}) is equal to 800 rpm. SME is therefore calculated according to Eq. (1).

$$\mathbf{SME = \frac{P}{Q_S} \text{ with } P = U \times I \times \cos\varphi \times \frac{S_S}{S_{max}}} \quad \mathbf{(1)}$$

With:

- P is the electrical power supplied by the motor (W).
- U is the operating voltage of the motor (V).
- I is the current consumed by the motor (A).
- cos φ is the theoretical efficiency of the motor.
- S_S is the screw speed (rpm).
- S_{max} is the maximum screw speed (rpm).
- Q_S is the inlet flow rate of the solid mixture (kg/h).

Extruded pellets were then collected and transferred to the dryer. This step was carried out using a continuous Clextral (Firminy, France) Evolum 600 belt dryer. Drying was conducetd at 120°C during 1800 sec for all the productions. The purpose of reducing moisture content was to make extruded pellets self-stable until the coating step. Pellets were then stored in a dry place (with final moisture content of around 6 % to 8 %).

Fat was exclusively added during the coating step, meaning that no oil was injected in the extruder at the moment of the extrusion cooking one. A Stolz (Paris, France) MRSV 100 pilot vacuum coater machine was used to conduct this task in order to raise the energy content of extruded pellets. An appropriate nozzle (APSIS 15/40) was chosen to guarantee an efficient spraying system. This configuration was optimal to spray the oily liquid uniformly. Firstly, the oil mixture composed of R, F1 and F2 oils (75/21/4 (w/w)) was prepared in a bucket and mixed intensely. The latter was then introduced into the oil tank and heated at 40 ± 2 °C. During the oil mixture heating, the extruded pellets were loaded inside the vacuum coater tank. Simultaneously with the oil tank's pressurization up to 3 bars, a vacuum pump was used to generate vacuum in the inside of the mixing vessel. This vacuum pump was a Busch (Gerlingen, Germany) Mink MM 1104 BV 1.3 kW one. Then, a spray nozzle allowed the oily blend to be injected progressively. It was positioned in the top of the coater tank. Thanks to this spray, a proper dispersion was realized onto the feed while the two mixing blades were rotating. The proportion between extruded pellets and the oily blend was 73/27 (w/w), thus enabling to reach the targeted energy content of the coated pellets (*i.e.*, 30 % (w/w) fat content). The coater's pressure then got back to the atmospheric pressure once the oil was fully distributed. Lastly, the coater overturned, and the coated pellets could thus be recovered. The coating conditions used in the present work have been determined from a previous study **(Chaabani et al., 2020)**. Identified as the optimal ones, they were as follows:

- 140 mbars for the vacuum coater's pressure at the moment of the oily blend addition.
- 65 Hz for the stirring speed.
- 45 % for the volumetric filling rate of the coater.
- 120 s for the time to restore the atmospheric pressure after the oily blend was added.

From each formulation, a sample of about 3 kg of coated pellets was collected after the coater's unloading, and these were then stored at 5 °C before their characterization. By taking such a precaution, the oil leakage has been avoided. NIR (Near-infrared) spectroscopy was used for all the experiments to ensure the 30 % (w/w) fat content in the coated pellets.

Considering that our objective was to understand specifically the role of the preconditioner in the *Fish Feed* process, extruded pellets were obtained as follows (**Table 2**):

- **Production of extruded pellets with preconditioner:** formulations S1 and S2, at 425 rpm and 575 rpm, respectively.

- **Production of extruded pellets without preconditioner:** formulations S3 and S5 at 425 rpm, and formulations S4 and S6 at 575 rpm. Additionally, two different amounts of water added in the twin-screw extruder were tested, *i.e.*, 26.5 kg/h for the S3 and S4 formulations, and 30.0 kg/h for the S5 and S6 formulations, corresponding to water-to-solid ratios of 0.241 and 0.273, respectively. On the one hand, for the S3 and S4 formulations, the 26.5 kg/h water flow rate corresponded to the sum of the Q_{PREC} and Q_{TSE} water flow rates used for the S1 and S2 formulations. On the other hand, in the case of the S5 and S6 formulations, the additional amount of water added (*i.e.*, 3.5 kg/h) corresponded to the amount of steam actually adsorbed by the solid mixture during the production of formulations S1 and S2.

2.4. *Fish Feed* pellet's usage properties

2.4.1. Bulk density and expansion ratio (ER)

Bulk density was determined by filling extruded pellets in a 1,000 mL cylinder and then determining the mass per unit volume of the sample. The pellets density was measured in triplicate and the result was expressed in g/L *(i.e.*, mass per volume) as mean values ± standard deviation. To standardize this measure, the cylinder was not tapped before weighing every sample **(Aas et al., 2011)**.

The diameter of twenty uncoated extruded pellets was measured with an electronic digital sliding caliper. This diameter was used to calculate the expansion ratio according to Eq. (2).

$$ER\ (\%) = \frac{\text{pellet diameter} - \text{die diameter}}{\text{die diameter}} \times 100 \qquad \textbf{(2)}$$

2.4.2. Durability

Pellet durability was determined using a Doris tester (Durability on a Realistic Test) from the AKVA Group (Klepp, Norway). Initially, a sample mass of 350 g of coated pellets was sieved with 1.0 mm and 7.1 mm screens. After sieving, the material collected on each screen was weighed. Dust was the quantity of material collected under the finest screen. Small fractures from broken pellets were the quantity of material between the two tested screens.

The Doris test was then applied on the only preserved pellets. The objective of this test was to simulate the stresses to which pellets are exposed to in pneumatic feeding devices **(Aas et al., 2011)**. Firstly, a known mass of preserved pellets was introduced on the Doris tester. The Doris device is made of an Archimede screw that feeds pellets from the inlet to the outlet of the tester. As the transportation can induce degradation by impact and shear, pellets were collected in a cup at the tester's outlet when the test was finished. Then, they were sieved with a 7.1 mm screen and the whole fraction of preserved pellets (collected on the upper screen) was weighed. The durability (expressed in % and possibly varying from 0% to 100%) was calculated as the ratio of the weight of preserved pellets to the initial weight of tested pellets. Extruded pellets of each formulation were analyzed in triplicate and results were expressed as mean values ± standard deviations.

2.4.3. Resistance to compression or hardness

Pellet hardness (H) was measured using an Instron 33R4204 (Norwood-Massachusettes, United States) universal testing machine fitted with a 5 kN load cell. Two rigid plates were used during the test with the analyzed pellet positioned between them. The crosshead speed was 2 mm/min at the moment of the compression test, and the test result was the required force (F) to have the pellet broken. Hardness (H) was then expressed as the ratio of the maximum force (F) to the average diameter of the analyzed pellet (d). Proceeding in this way guarantees that dimensional variations of pellets were taken into account. The reported values are therefore the average of twenty replications and results were expressed as mean values ± standard deviations.

2.4.4. Floatability

The floatability of each formulation was tested using a large glass beaker (18.5 cm in diameter) filled with 4 L of fresh water at ambient temperature. Using a small plastic shovel, fifty pellets were counted and were then thrown softly into the water beaker. The number of floating pellets that remained suspended in the water after 30 s was determined. In aquaculture, *Fish Feeds* are intended to either sink or float (depending on the fish species). That is why depending on the obtained floatability, pellets are classified as follows: sinking pellets (floatability < 30 %), semi-floating pellets (between 30 % and 70 %), and finally floating pellets (over 70 %). For each condition, the measurements were carried out three times and results were expressed as mean values ± standard deviations. The water was changed at the end of each replication.

2.4.5. Oil leakage rate (OLR)

Once coated, pellets tend to lose oil over time. The pellet's Oil Leakage Rate (OLR) was evaluated using a non-standardized method developed specifically for the purpose of this study. It was measured in two different conditions, *i.e.*, at 60 % relative humidity (RH) and 25 °C on the one hand, and at 40 °C on the other hand. Conducted in a climatic chamber, the first incubation condition corresponded to pellets stored in a controlled manner whereas the second one had the objective to simulate the high temperature to which the pellets may be exposed when stored in silos in full sun during the summer season. The latter was conducted in a ventilated oven.

For both tested incubation conditions, the test sample mass of pellets was 130.0 ± 0.1 g. These were then positioned in a glass beaker with a volume of 250 mL and at the bottom of which several layers of an absorbant paper, for a total mass of 2 g, have been placed. The incubation duration was chosen equal to 24 h. Specifically for the pellets incubated at 40 °C in a ventilated oven, the beaker has been left to cool to room temperature once the incubation period was over. Then, as part of the oil leaked during the test and has been absorbed by the paper, its weight was measured and this allowed determining the mass loss of the pellet in oil. Eq. (3) gives the formula used for the OLR calculation.

$$\mathbf{OLR\ (\%)} = \frac{\textbf{Weight of oil absorbed by the absorbent paper (g)}}{\textbf{Total weight of pellets before leakage (g)}} \times \mathbf{100} \qquad \mathbf{(3)}$$

Experiments were carried out in triplicate. The results were expressed as mean values ± standard deviations.

2.5.Starch gelatinization (SG)

To measure starch gelatinization rate, a Differential Scanning Calorimetry (DSC) apparatus was used. Firstly, test samples corresponding to the six uncoated pellets were obtained through a crushing step using a Nahita (La Chapelle sur Erdre, France) agate mortar with a 50 mm pestle. All the grinded powders were then conditioned in a climatic chamber (25 °C, 60 % RH) for three weeks to ensure an equivalent moisture content for all materials tested at the time of sampling. Lastly, samples were analyzed with a Mettler Toledo

(Columbus-Ohio, United States) DSC1 calorimeter under a constant flow of dry nitrogen. A hermetic steel sample pan with a volume of 100 µL was used for the purpose of the DSC analysis. It contained the sample to be analyzed, and the test sample mass was chosen around 10 mg. Then, the sample was completed with Milli-Q water (50 µL) and the analysis was realized after one night of incubation. After that, a heating ramp at 3 °C/min from 5 °C to 100 °C was performed.

The starch gelatinization rate was also determined on the starting solid mixture (S0) and on the mixture collected at the output of the preconditioner (*i.e.*, after having added water and steam to the starting solid mixture, and before reaching the twin-screw extrusion machine).

2.6.Morphological characterization of pellets through tomography

To better understand differences between the obtained pellets (S1 to S6), their morphology (internal structure (porosity) and oil distribution) were determined by tomography. The tomography device used to perform this analysis was a RX Solutions Easy Tom (Chavanod, France) 3D X-ray laboratory tomograph. For the analysis, a tension of 114 kV and a current of 263 µA were chosen. For each formulation, three pellets were analyzed. A rotating stage was used to support them. Approximately 1,440 projections of the transmitted X-ray intensity field were recorded for each angular step of 0.25°. This was made possible by the use of an X-ray detector and a flat panel detector (1,920 × 1,536 pixels). Then, a volume image of the analyzed sample could be reconstructed from all the radiographs using a filtered back-projection algorithm. This image allowed to observe, with a spatial resolution of 20 µm, the variations of the linear attenuation coefficient inside the sample.

Three different zones were distinguished in this reconstruction: the starchy matrix (in light grey), the pores filled by the oil (in dark grey), and the empty pores (in black). The AVISO software enabled the determination of the volume occupied by these three zones inside the coated pellets (V_{SM}, V_{PO} and V_{EP}, respectively), and the distribution in volume (% (v/v) in proportion to the pellet's total volume) could then be calculated. For each formulation, this quantitative analysis was conducted three times, and results were then expressed as mean values ± standard deviations.

From this distribution in volume, the pellet's porosity after extrusion-cooking and drying (*i.e.*, immediately before vacuum coating) was calculated according to Eq. (4).

$$\textbf{Pellet's porosity }(\%) = \frac{(V_{PO}+V_{EP})}{(V_{SM}+V_{PO}+V_{EP})} \times 100 \quad (4)$$

2.7. Water absorption and Water Solubility indices of extruded pellets

The uncoated extruded pellets were ground in a fine powder using a Foss (Hillerød, Denmark) Cyclotec 1093 grinder fitted with a 1 mm screen. About 2.5 g sample was suspended in 30 mL of distilled water in a 50 mL centrifuge tube. The suspension was stirred intermittently, placed in an oven at 30°C for 30 min and then centrifuged at 1000 × *g* for 10 min.

The supernatant liquid was decanted into an aluminium cup and then oven-dried for 2 h at 135 °C (AACC method 44-19, 1995). The weight of the gel remaining in the centrifuge tube was measured.

Water absorption (WAI, %) and water solubility (WSI, %) indices were subsequently calculated as follows:

$$\mathrm{WAI} = \frac{W_g}{W_{ds}} \times 100 \qquad (5)$$

Where:
W_g is the weight of the gel (g).
W_{ds} is the weight of the dry sample (g).

$$\mathrm{WSI} = \frac{W_s}{W_{ds}} \times 100 \qquad (6)$$

Where:
W_s is the weight of the dry matter in the supernatant (g).

2.8. Statistical analyses

Presented as mean values ± standard deviations, all data originated from triplicates, except hardness measurements that were performed on twenty pellets. The XLSTAT (Addinsoft, Bordeaux, France) software followed by a one-way analysis of variance (ANOVA) were used for analyzing the results in a statistical point of view so as to evaluate whether the difference between the pellet's physical properties is significant or not. $P < 0.10$ was chosen for each variable to assess the significance of the differences. Tukey's test was used in the multiple comparison procedure.

3. Results and discussion

3.1. Thermal analysis of the extruded pellets (evaluation of starch gelatinization)

The DSC analysis was used to estimate the starch gelatinization rate inside the solid mixture at the preconditioner outlet just as inside the extruded pellets before coating. Although it is not the only analytical method known for measuring the gelatinization rate of starch, the DSC analysis is suitable when the fat content of the analyzed samples is low. Otherwise, interactions between starch and fat make it difficult to observe the gelatinization phenomenon. In the present study, as fish oils (F1 and F2) and rapeseed oil (R) were added exclusively during the coating step, the uncoated pellets only contained the oily molecules coming from the initial solid mixture at the moment of their DSC analysis. This content was only 4.2% of the fresh matter, which made DSC analysis appropriate in this case.

The results obtained from the extruded pellets processed at 575 rpm (formulations S2, S4 and S6) are presented in **Fig. 3**, and particular attention has been focused on the temperature zone between 55°C and 80°C, where gelatinization of starch occurs. No thermal phenomenon was observed for these three formulations, and the same was true for the three others. This indicates that the starch gelatinization was complete during the extrusion process even in the case of formulations S3 to S6 for which no preconditioner was used. On the contrary, when conducted on the starting solid mixture (S0) or the preconditioned one (S_{2PREC}), DSC thermograms revealed a specific endothermic peak from 60 °C to 80 °C, which was characteristic of the starch gelatinization and thus evidencing that gelatinization was not complete inside the preconditioned mixture **(Fig. 3)**. **Table 3** gathers the starch gelatinization enthalpy (ΔH) for all the tested samples, and these enthalpy values were used to calculate the gelatinization rate (GR) of all processed mixtures (*i.e.*, the preconditioned ones and the extruded pellets) according to Eq. (7). ΔH (expressed in J/g) was determined by calculating the area under the DSC normalized peak.

$$\mathbf{GR\ (\%)} = \frac{\Delta\mathrm{H\ (initial\ mixture)} - \Delta\mathrm{H\ (processed\ mixture)}}{\Delta\mathrm{H\ (initial\ mixture)}} \times \mathbf{100} \quad (7)$$

A gelatinization rate of about 60 % was observed for the two preconditioned mixtures at the origin of the S1 and S2 formulations. This result is in perfect agreement with data in the literature reported by **Lavocat and Raymond (2018)**, which confirms the preconditioner role in aquafeed manufacturing. Raw materials (starch, protein, etc.) are hydrated and heated firstly in the preconditioner. A pre-cooking step is realized in the presence of water (in its steam and liquid forms), contributing to the loss of starch crystallinity and to the beginning of its gelatinization. Then, the pre-heated and pre-humidified mixture passes to the extruder, where the thermo-mechanical treatment is achieved by applying a temperature constraint along the barrel simultaneously with intensive shear stress resulting from the restrictive reverse elements positioned along the screw profile. In the literature, it was previously mentioned by **Riaz and Rokey (2011)** that the combination of generated heat, pressure and moisture inside the twin-screw extruder achieves cooking, starch gelatinization, and protein denaturation. **Hansen et al. (2010)** and **Kraugerud et al. (2011)** had reported that starch gelatinization varied between 73 % and 100 % in extruded *Fish Feed* and, this finding is in perfect accordance with the present DSC results that have revealed a complete gelatinization of starch for formulations S1 and S2.

Even without a preconditioning step, the extruder was still able to fully gelatinize starch, both with less (S3 and S4 formulations) or more (S5 and S6 formulations) water injected into the barrel. For aquafeed, starch acts as a natural binder contributing to the pellet's cohesion, a source of energy and a density control agent **(Bortone, 2017)**. To make this last capacity effective, the starchy fraction must be well cooked and gelatinized during the extrusion process. At this level, all the extruded pellets produced in this study fulfill this objective. Nevertheless, their usage properties must be determined and discussed in a comprehensive manner before definitively validating that the production of *Fish Feed* pellets can be satisfactory ensured without using a preconditioner.

3.2.Effect of the process parameters on pellet's qualities

As described previously, S1 and S2 formulations were obtained *via* the traditional *Fish Feed* process including a preconditioning step before extrusion **(Fig. 2)**. The related extrusion parameters, and pellet's characterizations and usage properties are summarized in **Table 4**. The moisture content is of critical importance to authorize efficient starch gelatinization and protein denaturation and, consequently, to maintain the nutritional quality during extrusion-cooking. It was measured at different levels during the *Fish Feed* process, *i.e.*, in the starting mixture, at the preconditioner outlet when used, at the extruder outlet and finally at the dryer outlet. The initial solid mixture had a moisture content equal to 7.4 ± 0.1 %. The latter stayed in the preconditioner for 3 to 4 min where it was pre-humidified *via* water and steam addition. This contributed to an increase in its moisture content to 19 % on average. In parallel, the particularly low associated standard deviation (0.2 % max) also indicates that water was uniformly distributed inside the solid mixture. The pre-humidified and pre-heated mixture left the preconditioner with an average temperature of 75 ± 2 °C. Therefore, moisture and temperature data confirm the preconditioner role, which indeed consists in pre-heating and pre-humidifying the solid mixture, to ensure the starch pre-gelatinization.

At the extruder level, the screw speed was the only variable operating parameter, differing between the S1 and S2 formulations (425 rpm and 575 rpm, respectively). The moisture content was also determined at the extruder outlet, and it was equal to 25 % on average, due to the additional water injected into the extruder. In fact, a moisture content between 25 % and 30 % during wet extrusion of diets involving a mixture of cereal products, and vegetable and animal proteins has been recommended for aquafeed **(Rokey, 1994)**. In such humidifying conditions, the formulation is better processed to obtain feed with high physical quality. Complete and uniform moisture penetration in the ingredient particles results in an increased heat transfer, allowing uniform gelatinization **(Rokey, 1994)**.

During the extrusion process, the electrical energy is converted to mechanical energy through the screws, and it is then transferred to the solid mixture. Representing the amount of mechanical energy input per mass unit, SME values thus appear as a good way to characterize the efficiency of the extrusion process. In this study, increasing the screw speed from 425 rpm to 575 rpm (whatever the formulation) enhanced shear stress, increasing the energy input. At a constant temperature, SME is known to increase with the screw speed **(Baik et al., 2004)**. An increased speed elevates the friction between the product and the screws: more mechanical energy is thus produced. Simultaneously, increasing the screw speed slightly promoted pellet's expansion observed through a decrease in its bulk density, confirming that it is an influential variable in twin-screw extrusion **(Rokey, 1994)**. It should however be noted here that despite the increase in both pellet's diameter and expansion ratio from formulation S1 to formulation S2, the values obtained were not different in a statistical point of view **(Table 4)**. Concerning the counter-pressure, which is also an important parameter that must be controlled during the extrusion process, and according to the values reported in **Table 4**, it decreased as the screw speed increased. The counter-pressure was in fact directly linked to the device's filling coefficient: the more the filling of the extruder, the more the counter-pressure.

With a reduced screw speed, the twin-screw machine was more filled and the counter-pressure logically increased.

Differences were found between the S1 and S2 formulations concerning their usage properties. Although slightly lower, the expansion level generated in S1 was a little more favorable to better retain oil in these pellets as confirmed by the lower OLR values obtained at 25 °C and 40 °C as compared to S2 **(Table 4)**. On the other hand, as S2 had a greater expansion ratio (55 % instead of 51 %), its durability and hardness were slightly reduced. In aquafeed, the expansion is one of the most important factors as it affects the density, hardness, brittleness and oil holding capacity **(Rosentrater et al., 2009)**. A large number of studies had reported that an inverse relationship existed between the pellet's expansion and physical quality parameters like durability and hardness **(Aarseth et al., 2006a; Hansen and Storebakken, 2007; Morken et al., 2012; Sørensen et al., 2009, 2010, 2011)**.

Fish Feed pellets must adapt to the species, and they are intended to either sink or float **(Dethlefsen, 2017)**. In our case, S1 gave semi-floating pellets while S2 gave fully floating ones. In fact, floatability was directly linked to the pellet's density after vacuum coating: the lower the density, the higher the floatability. This is the reason why the bulk density of coated pellets is also of key importance with regard to their floatability **(Chevanan et al., 2007, 2009)**. Bulk density is directly linked to the expansion degree of pellets generated during the extrusion process **(Glencross et al., 2011)**. In aquaculture, bulk density is not only adjusted according to the feeding habits of the targeted fish species but also to fish farmers feeding practices **(Sørensen, 2012)**.

The quality of pellets was also investigated for the S3 to S6 formulations obtained without the preconditioner. All the tested conditions allowed to obtain extrudable, pelletable and coatable *Fish Feed* (up to 30% (w/w) fat). According to the results presented in **Table 4**, S3 and S4 were the formulations with the lowest moisture contents at the extruder outlet (19.8% in average) of the entire study, and this had led to the pellets with the poorest usage properties. Firstly, both S3 and S4 pellets had a rough surface. This was the consequence of their low moisture contents, causing more difficulty for the extruded material to pass through the die orifice. In such conditions, and as water is known to act as a plasticizer for starch and proteins, the reduced moisture content increased the material viscosity, rendering less favorable its flowing. In addition, bonds (*i.e.*, hydrogen ones and/or van der Walls forces) may also appear between particles, due to moisture acting as a binder **(Pietsch, 2002)**. This property was, therefore, less favored in the case of the S3 and S4 formulations given their low moisture content at the extruder outlet. This resulted also in higher SME values **(Table 4)**. It should be remembered here that water is the most important and widely used plasticizer in extrusion processing **(Alvarez-Martinez et al., 1988; Blanche and Sun, 2004)**. S3 and S4 formulations had so higher bulk densities and reduced expansion ratios of only 32% and 36%, respectively. In fact, the expansion is highly affected by the flour moisture **(Draganovic et al., 2011)** and the driving force in the pellet's expansion is the vaporization of water **(Cheng and Friis, 2010)**. Thus, it is reasonable to deduce that a high content of moisture boosts the expansion potential. As the expansion affects the oil holding capacity **(Rosentrater et al., 2009)**, higher values of OLR at 25 °C and 40 °C were logically obtained for the less expanded pellets of the S3 and S4 formulations. Broken pellets and dust **(Table 4)** were also more important with S3 and S4. Low moisture content during extrusion was responsible of poorer cooking quality, therefore resulting in more fragile pellets (*i.e.*, with little cohesion and binding strength). These results are in perfect agreement with those reported by **Rokey (1994)**.

On the other hand, higher durability was obtained with S3 in comparison to S1. With less water and a low screw speed, S3 had a high density reflecting a less porous structure. It is reasonable to assume that this configuration explains the high durability of the S3 pellets. On the contrary, pellets from S4 had lower durability than those from S2 despite their lower moisture content, and this was probably the consequence of the higher screw speed chosen (575 rpm instead of 425 rpm). S3 and S4 had also lower hardness values in comparison with S1 and S2. The pellets obtained in that case were less homogeneous with non-uniform cooking, due to less water during the extrusion process, probably leading to more fragile pellets from a mechanical point of view. **Pietsch (1983)** had reported that water in pellets bound particles together by a "liquid bridge". This deficit in water during the extrusion of pellets from the S3 and S4 formulations could be responsible for their brittleness as the particles inside the mixture were less bound together during the extrusion process. Finally, S3 and S4 pellets were both sinking ones. Even increasing the screw speed (case of the S4 formulation) was not sufficient to have at least semi-floating pellets.

To conclude, S3 and S4 pellets did not meet the specifications of adult Trout feed. Even by increasing the screw speed (S4 formulation), such setting was not enough to obtain a floating pellet (floatability of 25% for a recommendation of at least 70 %). This difficulty to obtain pellets with the targeted properties can be explained by the fact that S3 and S4 were obtained in non-optimal moisture level conditions as found in the literature (25-30 %).

In addition to these poor usage properties, pellets from S3 and S4 could present other disadvantages due to their low moisture content. For example, **Rokey (1994)** had reported that the palatability was reduced when lower moisture levels were applied. This condition can even contribute to the destruction of heat-labile nutrients like lysine and ascorbic acid. The same author recommended a moisture content of 250-300 g/kg during the extrusion of fish diets to prevent losses of nutrients. In our case, this moisture content was only 241 g/kg for both S3 and S4 formulations **(Table 2)**. **Finley (1989)** and **Opstvedt et al. (1984)** have also found that when low moisture content occurred in combination with temperature higher than 100 °C, the cross-linking between amino acids could occur inside the fish proteins. Consequently, digestibility was reduced for almost all amino acids, especially cysteine **(Andorsdottir, 1985; Ljøkjel et al., 2000)**. The same phenomenon was observed with rainbow Trout when water added to the extruder was restricted in comparison with diets obtained at more elevated moisture contents **(Sørensen et al., 2002)**. In other terms, in the case of the S3 and S4 formulations and apart from the fact that they were obtained without preconditioner, their processing with low moisture content could affect negatively their digestibility.

Since S3 and S4 resulted in pellets with unsatisfactory properties, corrective measures were applied by injecting more water inside the extruder barrel (30.0 kg/h instead of 26.5 kg/h) when extruding the S5 and S6 formulations without using the preconditioner. In fact, this correction was made by taking into account the water that was absorbed by the solid mixture in the preconditioner in the case of formulations S1 and S2. This adjustment was evidenced by higher moisture content values at the extruder outlet for S5 and S6 in comparison with S3 and S4: 24 % instead of 20 % **(Table 4)**. The most important finding is that adding more water had significantly improved the pellet's usage properties, which became then similar to S1 and S2, especially with the highest screw speed (575 rpm) **(Table 4)**, confirmed by the statistical analysis. Adding more water was necessary for the viscoelastic properties of the extruded mixture and to guarantee complete hydration. S5 and S6 densities (both at the extruder outlet and at the coater one) were reduced and became much closer to S1 and S2 ones. In this case, water injected in the extruder barrel can be used to control the extruded pellet's density **(Bortone, 2017)**. Consequently, more water helped to generate more expanded pellets with a larger diameter that met the expansion level of S1 and S2 (with no statistical difference). This improvement contributed also to better oil retention, which resulted in lower OLR values at 25 °C, compared to S3 and S4, in the range to those measured for S1 and S2.

At 40 °C, no statistical difference was observed for OLR of pellets extruded at 575 rpm (comparison between S2 and S6). In fact, the combined action of an additional amount of water and an increase in the screw speed allowed a further improvement on the pellet's usage properties, explaining why the results for S6 were so close to those for S2. Additionally, floating pellets were obtained with S6 (86 %). Despite a slight floatability reduction in comparison with S2, S6 still met the feed specifications for large Trout (*i.e.*, floating pellet type). Dust generation was also not statistically different between S2 and S6, and particularly limited. These results demonstrate the beneficial effect of adequate moisture content during extrusion to generate desirable usage properties even without the preconditioning step.

Overall, durability and hardness were improved with S5 and S6 compared to S1 and S2. Such improvement in the pellet's usage properties is often desired in aquaculture. In this situation, the ability of the feed to be handled while maintaining its integrity (fewer dust amount) is guaranteed **(Sørensen et al., 2012)**. In intensive aquaculture (*e.g.*, big and modern Trout farming), feeds should be resistant to a multitude of constraints such as handling, mechanical stress during transport and pneumatic feeding devices **(Aarseth, 2004; Aarseth et al., 2006b)**. That is why the durability measurements made at lab scale (use of a Doris tester) tend to mimic the stress applied to the pellets in real conditions. On the other hand, hardness assesses the resistance to breakage when pellets are exposed to external pressure. It is often used to mimic the force applied on pellets when they are stacked on top of each other during storage in silos or bins, the mechanical solicitation in a screw conveyor, and the compressing force between animal teeth **(Kaliyan and Morey, 2009)**.

In the specific case of diets for salmonids, starch is kept at a lower concentration than proteins, due to their low capacity to digest and metabolize starch **(Hemre et al., 2002)**. As native proteins generally have a stronger water affinity than starch **(Semenova et al., 2006; Yahata et al., 2006)**, it is reasonable to assume that water will be more easily absorbed by the proteins inside the extruder when no preconditioner is used. Additionally, and for the same reason, adding more water to the mixture in the extruder (S5 and S6 formulations in comparison with the S3 and S4 ones) must undoubtedly have contributed to an increased absorption of water by the proteins and thus their improved denaturation, which must be the reason why the pellet's durability and hardness were improved for the S5 and S6 formulations. These results underline the twin-screw extruder ability to ensure the entire *Fish Feed* process, *i.e.*, both the starch gelatinization as previously evidenced by the DSC results **(Table 3** and **Fig. 3)** and the protein denaturation.

Besides, it is well known that high water content during extrusion contributes to higher nutrient availability **(Sørensen, 2012)**. For example, when shrimp were fed by extruded diets at high moisture content rather than from dry extrusion conditions, the results showed an improvement in growth performance that emphasizes the importance of moisture during processing **(Obaldo et al., 2000)**. Given these results, it appears therefore necessary to complete the present study by *in vivo* feeding trials on adult Trout in order to check if those findings will still be valid in the absence of preconditioner. As a more elevated water content during extrusion was used for S5 and S6, these two formulations should be the most interesting ones in terms of palatability and nutrient digestibility, especially since the pellets from these two formulations have a common appearance once coated. Indeed, **Fig. 4** shows the extruded pellets obtained at a screw speed of 575 rpm before and after coating. No visual difference was observed between S2 and S6. On the contrary, the S4 pellets were distinguishable from the two others as they revealed a less uniform appearance and a rougher surface.

To conclude, a complete starch gelatinization can occur along the twin-screw extruder barrel alone without any preconditioning treatment. In addition, the same level of expansion can be reached at the die outlet but on the express condition that the liquid water injected in the extruder barrel is the sum of both liquid water flow rates (*i.e.*, the one added in the preconditioner and the second in the twin-screw extruder) and the part of injected steam really absorbed by the flours along the preconditioner when the latter is implemented. For future work, it will be however necessary to ensure that not using a preconditioner will not lead to a premature wear of both the screw elements and the barrel in the twin-screw machine.

3.3.Morphological characterization of pellets through tomography

Results obtained for the pellet's expansion ratios, and their density, OLR values and floatability were completed by their morphological characterization through 3D X-ray tomography. **Fig. 5** presents one example of tomography image taken from all coated pellets analyzed (S1 to S6). Thanks to tomographic reconstructions of the extruded pellets, it was possible to carry out a quantitative analysis that enabled to determine the distribution in volume relative to the three different zones inside the coated pellets (*i.e.*, the starchy matrix, the oil-filled pores and the empty ones).

Table 5 shows significant differences in the volume distribution between these three different zones when comparing the pellets produced with preconditioner (S1 and S2) and those without (S3 and S4). In particular, the S3 and S4 formulations produced without preconditioner and with a reduced amount of water revealed a reduced proportion of oil-filled and empty pores. On the contrary, when more water was added in the extruder (S5 and S6), these differences were largely reduced and the volumes occupied by the oil-filled and empty pores were not statistically different from those inside the S1 and S2 pellets. These tomography results are very relevant since the same trend was observed previously in terms of usage properties. With the lowest moisture content (S3 and S4), results in **Table 4** showed that expansion was unfavored, resulting in higher OLR values (especially at 25 °C). The obtained pellets were therefore less porous (higher density), logically explaining at the same time the much higher values for V_{SM}. However, even without a preconditioner but with the express condition of adding higher proportion of water in the extruder barrel, the obtained pellets (S5 and S6) presented an internal structure similar to those obtained with the preconditioned mixtures. It is thus reasonable to assume that the expansion phenomenon generated at the die outlet was equivalent between the S1 and S2 formulations on the one hand, and the S5 and S6 ones on the other hand.

Pastor-Cavada et al. (2011) had reported that bulk density and expansion ratio were the best properties to describe product porosity. In this context and to verify the previous hypothesis, the sum of V_{PO} and V_{EP} was used to determine the pellet's porosity **(Table 4)**. The results revealed no statistical difference between S1, S2, S5 and S6 pellets. These findings are in perfect agreement with the previously discussed results related to the expansion ratio. On the other hand, S3 and S4 had the lowest pellet's porosity, reflecting their lower expansion ratio, which was the consequence of the reduced water amount injected inside the extruder barrel. These results highlight the clear correlation existing between the pellet's expansion ratio and their porosity.

To conclude, the expansion ratio generated during extrusion strongly influences the usage properties and final appearance of the product **(Moraru and Kokini, 2003)**. In the literature, the screw speed, the shape and the diameter of the die are known to influence expansion. In this study, even without a preconditioning step, it has been shown that expansion could be also adjusted thanks to the judicious choice of the quantity of added water. Without preconditioner, an adequate optimization of the extrusion-cooking conditions can thus perfectly lead to pellets as efficient as those obtained through a classical *Fish Feed* process. The key element is the expansion phenomenon that dictates the usage properties (*i.e.*, density, OLR and floatability) of the final extruded pellets.

3.4. WAI and WSI values

In several studies, WAI and WSI indices are often used to provide further information about the cooking rate of starch. This method is particularly justified at a practical level, as it does not require significant time, biochemical extraction, and isolation and characterization of a complex mixture **(Kim et al., 1989; Kirby et al., 1988)**. The values of WAI and WSI measured on the preconditioned mixture and extruded pellets are summarized in **Table 6**.

Kirby et al. (1988) indicated that a degradation of molecules could be illustrated through WSI values. As starch degrades, the quantity of soluble matters increases, and this results in modifications in the WSI value **(Guha et al., 1997)**. According to **Table 6**, no statistical difference in terms of WSI values was evidenced for the six extruded formulations and even for the preconditioned mixture. Alternatively, **Seker (2005**) and **Van den Einde et al. (2003)** have reported that high WSI values are the indication of effective starch gelatinization. In our case, this information means that starch gelatinization was the same for S1 to S6 formulations. This finding is consistent with the previous DSC results. And, although the differences were not significant in a statistical point of view, the lowest WSI value was obtained with the preconditioned mixture for which the gelatinization rate was estimated through DSC analysis to only 60% **(Table 3)**. This result can be explained as the latter had only undergone preconditioning while pellets have been extruded, with much higher mechanical shear applied.

In an aqueous dispersion, **Mason and Hoseney (1986)** indicated that it is possible to estimate the volume of gelled and swollen particles that retain their integrity *via* the WAI value. According to **Table 6**, no statistical difference was found between the S1 and S2 pellets (with preconditioner), and the S5 and S6 ones (without preconditioner and with a higher amount of water added). **Bortone (2004)** and **Gomez and Aguilera (1984)** had reported that WAI is related to starch gelatinization extent followed by degradation and dextrinization. These phenomena are responsible for water absorption. On the other hand, WAI values relative to pellets S3 and S4 were statistically different in comparison with those of S5 and S6. When the moisture content in pellets was reduced (S3 and S4), starch swelling was therefore limited in comparison with formulations also obtained without preconditioner but with more added water during the process (S5 and S6). This result is consistent with previous studies conducted by **Anderson et al. (1970)** and **Oikonomou and Krokida (2012)**, where WAI had decreased when the moisture content was reduced, but reported as well that WAI decreased with an increase in screw speed (no correlation established in this work).

Ravindran et al. (2011) have reported that WSI reflects the pellet's hydrophobicity while WAI indicates their hydrophilic capacity. These two parameters are useful as they can therefore influence the pellet's usage properties. In the present study, WAI and WSI values relative to S1 and S2 were not statistically different from those of S5 and S6. This finding was confirmed by the results presented in **Table 4** where most of the pellet's characteristics were kept without preconditioner with the precaution to work with adequate moisture content in the extruder.

The starch contained in pellets consists of two chains of glucose molecules, namely amylose and amylopectin. The first one is a linear polymer with a few branches while the second one is a highly branched polymer **(Tharanathan, 1995)**. The extrusion process of pellets causes substantial changes in the physical constitution of the starchy fraction **(Davidson et al., 1984)**. Those changes confer specific functional properties to the *Fish Feed* pellets. Among the most important practical properties of starch, its ability to swell, contributes to viscous paste, either in hot water or when treated with chemicals **(Knight, 1969)**. It is also important to specify that other components like proteins and fats can also influence the starch conversion during extrusion **(Lin et al., 1997)**. To conclude this part, it should be kept in mind that results concerning WAI and WSI are not based on fundamental structural characteristics of the material. This is the reason why discussing them has been done cautiously in the present study.

In aquaculture, *Fish Feed* pellets are very often produced using a preconditioner that precooks the feed (pre-gelatinization of starch). Not using it requires consequently subtle changes, specifically at the extruder level. The present work demonstrated that an optimization was necessary to achieve the desired product attributes while maximizing its usage properties. In our case, as pellets are designed to feed large Trout, it was necessary to manufacture them at high moisture content and high screw speed in the process without preconditioning. The adjustment of other extrusion parameters (*e.g.*, screw profile, temperature profile, etc.) would also possibly enable to reach the pellet's targeted properties. Further research will be required to determine the importance of each of them.

4. Conclusion

In this work, *Fish Feed* pellets for adult Trout were successfully manufactured at pilot scale through extrusion cooking without a preconditioner. The gelatinization of starch can be entirely conducted inside the twin-screw machine, thus reducing the investment cost of the industrial lines. The simplification of the process results as well in pellets with the same usage properties as those originating from a classical *Fish Feed* process including both a preconditioner and a twin-screw extruder. A final check of screw elements and extruder barrel wear would however be necessary to ensure the industrial gain of this simplified process over the long term. Indeed, even if the specific mechanical energy required was almost the same for both processes, the counter-pressure was significantly higher without the preconditioner. For future work, as the process without preconditioner has been validated in terms of pellet's usage properties, it will be necessary to carry out *in vivo* feeding trials on adult Trout to evaluate the pellet's palatability and nutrient digestibility, as these two characteristics are the most reliable barometers of product quality.

CRediT authorship contribution statement

Asma Chaabani: Investigation, Writing - original draft, Writing - review & editing, Visualization. Laurent Labonne: Investigation. Vanessa Durrieu: Formal analysis, Writing - original draft, Writing - review & editing. Antoine Rouilly: Writing - original draft, Writing - review & editing. Fabien Skiba: Resources, Supervision, Project administration. Philippe Evon: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

This research was granted by ANRT (National Association for Research and Technology in France). We would like to thank also Dr. Marina Fazzini (Laboratoire Génie de Production, ENI, Tarbes, France) for conducting the morphological characterization of pellets through tomography.

References

AACC. 44-19. 1995. Moisture-air oven method, drying at 135 °C. AACC Approved methods (9th ed.). St. Paul, MN, USA.

Aarseth, K.A., 2004. Attrition of feed pellets during pneumatic conveying: the influence of velocity and bend radius. Biosyst. Eng. 89, 197-213. https://doi.org/10.1016/j.biosystemseng.2004.06.008

Aarseth, K.A., Sørensen, M., Storebakken, T., 2006a. Effects of red yeast inclusions in diets for salmonids and extrusion temperature on pellet tensile strength: Weibull analysis. Anim. Feed Sci.Tech. 126, 75-91. . https://doi.org/10.1016/j.anifeedsci.2005.06.005

Aarseth, K.A., Perez, V., Bøe, J.K. Jeksrud, W.K., 2006b. Reliable pneumatic conveying of . Aquacult. Eng. 35, 14-25. . https://doi.org/10.1016/j.aquaeng.2005.06.006

Aas, T. S., Oehme, M., Sørensen, M., He, G., Lygren, I., Åsgård, T., 2011. Analysis of pellet degradation of extruded high-energy fish feeds with different physical qualities in a pneumatic feeding system. Aquacult. Eng. 44, 25-34. https://dx.doi.org/10.1016/j.aquaeng.2010.11.002

Altan, A., McCarthy, K.L., Maskan, M., 2009. Effect of screw configuration and raw material on some properties of barley extrudates. J. Food Eng. 92, 377-382. https://doi.org/10.1016/j.jfoodeng.2008.12.010

Alvarez-Martinez, L., Kondury, K.P., Harper, J.M., 1988. A general model for expansion of extruded products. J. Food Sci. 53, 609-615. https://doi.org/10.1111/j.1365-2621.1988.tb07768.x

Anderson, R. A., Conway, H., Peplinski, A. J., 1970. Gelatinization of corn grits by roll cooking, extrusion cooking and steaming. Starch-Stärke 22 (4), 130-135. https://doi.org/10.1002/star.19700220408

Andorsdottir, G., 1985. PhD. Protein quality, methionine supplementation and fat levels in starter diets for salmon fry. Cand. Scient. University of Oslo, p. 109.

Baik, B. K., Powers, J., Nguyen, L. T., 2004. Extrusion of regular and waxy barley flours for production of expanded cereals. Cereal Chem. 81, 94-99. https://doi.org/10.1094/CCHEM.2004.81.1.94

Bell, G.J., Koppe, W., 2010. Lipids in Aquafeeds. In: Turchini, G.M., Ng, W.K., Tocher, D. R. (Eds.), Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press, Boca Raton, pp. 21-59.

Blanche, S. & Sun, X., 2004. Physical characterization of starch extrudates as function of melting transitions and extrusion conditions. Adv. Polym. Technol. 23, 277-290. https://doi.org/10.1002/adv.20017

Bortone, E. J., 2004. Extrusion processing part I. Ingredient functionality of formulas. Global Aquaculture Advocate. 7, 69-72. Retrieved from https://www.aquaculturealliance.org/advocate/extrusion-processing-of-aquatic-feeds-part-1/ (accessed 25 January 2021).

Bortone, E., 2006. Vacuum Process Supports High Oil Content in Feed Coatings. Global Aquaculture Advocate, July/August 2006, pp. 68-69. Retrieved from: https://www.aquaculturealliance.org/advocate/vacuum-process-supports-high-oil-content-in-feed-coatings/ (accessed 30 January 2020).

Bortone, E., 2017. Performance of aquafeed ingredients in extrusion cooking. Global Aquaculture Advocate, October 2017. Retrieved from https://www.aquaculturealliance.org/advocate/preconditioning-crucial-aquafeed-manufacturing (accessed 14 March 2021).

Burel, C., 2017. Bases de la nutrition et formulation en aquaculture. Durabilité des aliments pour le poisson en aquaculture : réflexions et recommandations sur les aspects technologiques, économiques, sociaux et environnementaux, UICN - Union Internationale pour la Conservation de la Nature; Comité Français de l'UICN, 296 p., 2017, 9782831718316. Hal-01607152. https://doi.org/10.2305/IUCN.CH.2017.02.fr

Chaabani, A., Labonne, L., Tercero, C. A., Picard, J. P., Advenier, C., Durrieu V., Rouilly, A., Skiba, F., Evon, Ph., 2020. Optimization of vacuum coating conditions to improve oil retention in trout feed. Aquacult. Eng. 91, 102-127. https://doi.org/10.1016/j.aquaeng.2020.102127

Cheng, H., Friss, A., 2010. Modelling extrudate expansion in a twin-screw food extrusion cooking process through dimensional analysis methodology. Food Bioprod. Process. 88, 188-194. https://doi.org/10.1016/j.fbp.2010.01.001

Chevanan, N., Muthukumarappan, K., Rosentrater, K.A. & Julson, J.L., 2007. Effect of die dimensions on extrusion processing parameters and properties of DDGS-based aquaculture feeds. Cereal Chem. 84(4), 389-398. https://doi.org/10.1094/CCHEM-84-4-0389

Chevanan, N., Muthukumarappan, K. & Rosentrater, K.A., 2009. Extrusion studies of aquaculture feed using distillers dried grains with solubles and whey. Food Bioprocess. Technol. 2(2), 177-185. https://doi.org/10.1007/s11947-007-0036-8

Clayton, G., 2002. Better product density control. Feed International, 23(11), 4.

Craig, S., 2009. Understanding fish Nutrition, Feeds, and Feeding. VCE Publications, Virginia Tech. Retrieved from https://vtechworks.lib.vt.edu/bitstream/handle/10919/48950/420-256_pdf.pdf?sequence=1 (accessed 2 January 2020).

Davidson, V. J., Paton, D., Diosady, L. L., Larocque, G. J., 1984. Degradation of wheat starch in a single screw extruder: Characteristics of extruded starch polymers. J. Food Sci. 49, 453-458. https://doi.org/10.1111/j.1365-2621.1984.tb12441.x

Dethlefsen, M.W., 2017. PhD. Die Hard - improving the physical quality of extruded fish, feed pellets. Kgs. Lyngby: National Food Institute, Technical University of Denmark. https://backend.orbit.dtu.dk/ws/files/138706490/_public.pdf (accessed 26 January 2021).

Draganovic, V., Van der Goot, A.J., Boom, R., Jonkers, J., 2011. Assessment of the effects of fishmeal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed. Anim. Feed Sci. Technol. 165, 238-250. https://doi.org/10.1016/j.anifeedsci.2011.03.004

Finley, J.W., 1989. Effects of processing on proteins: an overview. In: Protein Quality and the Effects of Processing (Phillips, R.D. & Finley, J.W. eds), pp. 1-8. Dekker, New York.

Gaosong, J., Vasanthan, T., 2000. Effect of Extrusion Cooking on the Primary Structure and Water solubility of b-Glucans from Regular and Waxy Barley. Cereal Chem. 77, 396-400. https://doi.org/10.1094/CCHEM.2000.77.3.396

Glencross, B., Rutherford, N. & Hawkins, W., 2011. A comparison of the growth performance of rainbow trout (Oncorhynchus mykiss) when fed soybean, narrow-leaf or yellow lupin meals in extruded diets. Aquacult. Nutr. 17, e317-e325. https://doi.org/10.1111/j.1365-2095.2010.00765.x

Godavarti, S., Karwe, M.V., 1997. Determination of specific mechanical energy distribution on a twin-screw extruder. J. Agr. Eng. Res. 67, 277-287. https://doi.org/10.1006/jaer.1997.0172

Gomez, M. H., Aguilera, J. M., 1984. A physicochemical model for extrusion of cornstarch. J. Food Sci. 49, 40-43. https://doi.org/10.1111/j.1365-2621.1984.tb13664.x

Guha, M., Ali, S. Z., Bhattacharya, S., 1997. Twin-screw extrusion of rice flour without a die: Effect of barrel temperature and screw speed on extrusion and extrudates characteristics. J. Food Eng. 32, 251-267. https://doi.org/10.1016/S0260-8774(97)00028-9

Hansen, J.Ø., Storebakken, T., 2007. Effects of dietary cellulose level on pellet quality and nutrient digestibilities in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 272 (1-4), 458-465. https://doi.org/10.1016/j.aquaculture.2007.09.005

Hansen, J.Ø., Penn, M., Øverland, M., Shearer, K.D., Krogdahl, Ǻ., Mydland, L.T., Storebakken, T., 2010. High inclusion of partially deshelled and whole krill meals in diets for Atlantic salmon (*Salmo salar).* Aquaculture 310, 164-172. https://doi.org/10.1016/j.aquaculture.2010.10.003.

Hemre, G.I., Mommsen, T.P., Krogdahl, Ǻ., 2002. Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. Aquacult. Nutr. 8, 175–194. https://doi.org/10.1046/j.1365-2095.2002.00200.x

Kaliyan, N., Morey, R.V., 2009. Factors affecting strength and durability of densified products. Biomass Bioenerg. 33, 337–359. https://doi.org/10.1016/j.biombioe.2008.08.005

Khater, E.S.G., Bahnasawy, A.H., Ali, S.A., 2014. Physical and mechanical properties of fish feed pellets. J. Food Process. Technol. 5(10), 1000378. https://10.4172/2157-7110.1000378

Kim, C. H., Maga, J. A., Martin, J. T., 1989. Properties of extruded blends of wheat dried distiller grain flour with other flours. Int. J. Food Sci. Tech. 24, 373-384. https://doi.org/10.1111/j.1365-2621.1989.tb00657.x

Kirby, A. R., Ollett, A. L., Parker, R., Smith, A. C., 1988. An experimental study of screw configuration effects in the twin-screw extrusion cooking of maize grits. J. Food Eng. 8(4), 247-272. https://doi.org/10.1016/0260-8774(88)90016-7

Knight, J. W., 1969. The starch industry (1st ed.). New York, NY, USA: Pergamon. https://www.worldcat.org/title/starch-industry/oclc/706868822

Kraugerud, O.F., Jørgensen, H.Y., Svihus, B., 2011. Physical properties of extruded fish feed with inclusion of different plant (legumes, oilseeds, or cereals) meals. Anim. Feed Sci. Technol. 163(2-4), 244-254. https://doi.org/10.1016/j.anifeedsci.2010.11.010

Kraugerud, O.F., Svihus, B., 2011. Effects of online pretreatment of plant ingredients on processing responses and physical properties in extruded fish feed. Anim. Feed Sci. Tech. 168, 250-256. https://doi.org/10.1016/j.anifeedsci.2011.04.089

Lamichhane, S., Sahtout, K., Smillie, J., Scott, T.A., 2015. Vacuum coating of pelleted feed for broilers: opportunities and challenges. Anim. Feed Sci. Technol. 200, 1–7. https://doi.org/10.1016/j.anifeedsci.2014.11.015

Lavocat, E., Raymond, S., 2018. Preconditioning in Animal Feed Extrusion Processing. Animal Feed Extrusion Course organized by Clextral at Saint Etienne (LYON), October 16th, 2018.

Li, Y., Li, J., Liu, Z., Ruan, R., Mao, Z., 2003. Vacuum coating of heat-sensitive liquid ingredients onto feed pellets. Trans. ASAE. 46 (2), 383-387.

Lin, S., Hseih, F., Huff, H. E., 1997. Effect of lipids and processing conditions on degree of starch gelatinization of extruded dry pet food. LWT-Food Sci. Technol. 30, 754-761. https://doi.org/10.1006/fstl.1997.0271

Ljøkjel, K., Harstad, O.M., Skrede, A., 2000. Effect of heat treatment of soybean meal and fishmeal on amino acid digestibility in milk and dairy cows. Anim. Feed Sci. Technol. 84, 83-95. https://doi.org/10.1016/S0377-8401(00)00104-8

Maas, R.M., Verdegem, M.C.J., Stevens, T.L., Schrama, J.W., 2020. Effect of exogenous enzymes (phytase and xylanase) supplementation on nutrient digestibility and growth performance of Nile tilapia (*Oreochromis niloticus*) fed different quality diets. Aquaculture 529, 735723. https://doi.org/10.1016/j.aquaculture.2020.735723

Mason, W. R., Hoseney, R. C., 1986. Factors affecting the viscosity of extrusion cooked wheat starch. Cereal Chem. 63, 436-441.

Médale, F., Guillaume, J.C., 1999. Nutrition énergétique. *In :* Guillaume, J., Kaushik, S., Bergot, P., Métailler, R. (Eds), Nutrition des poissons et des crustacés, INRA, Paris, France : 87-111.

Mercier, C., Linko, P., Harper. J.M., 1989. Extrusion Cooking, p. 471. American Association of Cereal Chemists, St Paul, MN. https://doi.org/10.1002/star.19900420115.

Moraru, C.I., Kokini, J.L., Kokini 2003. Nucleation and expansion during extrusion and microwave heating of cereals foods. Compr. Rev. Food Sci. Food Saf. 2, 147-165. https://doi.org/10.1111/j.1541-4337.2003.tb00020.x

Morken, T., Kraugerud, O.F., Sørensen, M., Storebakken, T., Hillestad, M., Christiansen, R., Øverland, M., 2012. Effects of feed processing conditions and acid salts on nutrient digestibility and physical quality of soy-based diets for Atlantic salmon (*Salmo salar*). Aquacult. Nutr. 18(1), 21-34. https://doi.org/10.1111/j.1365-2095.2011.00872.x

Obaldo, L.G., Dominy, W.G., Ryu, G.H., 2000. Extrusion processing and its effect on aquaculture diet quality and shrimp growth. J. Appl. Aquacult. 10(2), 41-54. https://doi.org/10.1300/J028v10n02_04

Oikonomou, N. A., Krokida, M. K., 2012. Water absorption index and water solubility index prediction for extruded food products. Int. Food Prop. 15, 157-168. https://doi.org/10.1080/10942911003754718

Opstvedt, J., Miller, R., Hardy, R.W., Spinelli, J., 1984. Heat-induced changes in sulfhydryl groups and disulfide bonds in fish protein and their effect on protein and amino acid digestibility in rainbow trout (*Salmo gairdneri*). J. Agric. Food. Chem. 32(4) 929-935.

Pandey, B., 2018. Pellet Technical Quality of Feeds for Atlantic Salmon. Master's Thesis. Norwegian University of Life Sciences. Ås.-96.

Pastor-Cavada, E., Drago, S., González., R., Juan, R., Pastor, J., Alaiz., M., Vioque, J., 2011. Effects of the addition of wild legumes *(Lathyrus annuus and Lathyrus clymenum)* on the physical and nutritional properties of extruded products based on whole corn and brown rice. Food Chem. 128, 961-967. https://doi.org/10.1016/j.foodchem.2011.03.126

Pietsch, W., 1983. Agglomerate bonding and strength. In: Elements II. Briquetting and Agglomeration. Proceedings of the Institute for Briquetting and Agglomeration, Manitowish Waters, WI, USA (ed. by R.M. Koerner & J.A.MacDougall), pp. 9-40. Engineering Information, Inc., Hoboken, NJ, USA.

Pietsch, W., 2002. Agglomeration Processes: Phenomena, Technologies, Equipment (p. 622). Weinheim: Wiley-VCH. https://dx.doi.org/10.1002/9783527619801

Ravindran, G., Carr, A., Hardacre, A., 2011. A comparative study of the effects of three galactomannans on the functionality of extruded pea-rice blends. Food Chem. 124, 1620-1626. https://dx.doi.org/10.1016/j.foodchem.2010.08.030

Riaz, M.N., 2000. Extruders in Food Applications. CRC Press.

Riaz, M.N., 2019. Food extruders. In Handbook of farm, dairy and food machinery engineering (pp. 483-497). Academic Press. https://doi.org/10.1016/B978-0-12-814803-7.00019-1

Riaz, M.N., Rokey, G.J., 2011. Extrusion Problems Solved: Food, Pet Food and Feed. Woodhead Publishing Limited, Sawston.

Rokey, G.J., 1994. Pet food and fish food extrusion. In: The Technology of Extrusion Cooking (Frame, N.D. ed.), 1st edn, pp. 144-189. Blackie Academic & Professional, Glasgow, UK.

Rokey, G.J., 2006. Increasing Aquatic Feed Production through Plant Optimization, Wenger Manufacturing Inc. Publication, pp. 83-88.

Rosentrater, K.A., Muthukumarappan, K., Kannadhason, S., 2009. Effects of ingredients and extrusion parameters on aquafeeds containing DDGS and potato starch. J. Aquacult. Feed Sci. Nutr. 1, 22-38. https://medwelljournals.com/abstract/?doi=joafsnu.2009.22.38

Saalia, F. K., Phillips, R. D., 2011. Degradation of aflatoxins by extrusion cooking: Effects on nutritional quality of extrudates. Lwt-Food Sci. Technol. 44, 1496-1501. https://doi.org/10.1016/j.lwt.2011.01.021

Seker, M., 2005. Selected properties of native or modified maize starch/soy protein mixtures extruded at varying screw speed. J. Sci. Food Agr. 85, 1161-1165. https://doi.org/10.1002/jsfa.2086

Semenova, M.G., Belyakova, L.E., Polikarpov, Y.N., Il'in, M.M., Istarova, T.A., Anokhina, M.S., Tsapkina, E.N., 2006. Thermodynamic analysis of the impact of the surfactant–protein interactions on the molecular parameters and surface behavior of food proteins. Biomacromolecules 7(1), 101–113. https://doi.org/10.1021/bm050455m

Sørensen, M., 2012. A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. Aquacult. Nutr. 18, 233-248. https://doi.org/10.1111/j.1365-2095.2011.00924.x

Sørensen, M., Ljøkjel, K., Storebakken, T., Shearer, K.D. & Skrede, A., 2002. Apparent digestibility of protein, amino acids and energy in rainbow trout (Oncorhynchus mykiss) fed a fishmeal based diet extruded at different temperatures. Aquaculture 211, 215-225. https://doi.org/10.1016/S0044-8486(01)00887-0

Sørensen, M., Stjepanovic, N., Romarheim, O.H., Krekling, &Storebakken, T., 2009. Soybean meal improves the physical quality of extruded fish feed. Anim. Feed Sci. Technol. 149(1-2), 149-161. https://doi.org/10.1016/j.anifeedsci.2008.05.010

Sørensen, M., Luyen, G.Q.N., Storebakken, T. & Øverland, M., 2010. Starch source, screw configuration and injection of steam into the barrel affect physical quality of extruded fish feed. Aquacult. Res. 41(3), 419-432. https://doi.org/10.1111/j.1365-2109.2009.02346.x

Sørensen, M., Morken, T., Kosanovic, M., Øverland, M., 2011. Pea and wheat starch possess different processing characteristics and affect physical quality and viscosity of extruded feed for Atlantic salmon. Aquacult. Nutr. 17, e326-e336. https://doi.org/10.1111/j.1365-2095.2010.00767.x

Strahm, B.S., 2000. Preconditioning. In: Extruders in Food Applications (Riaz, M.N. ed.), pp. 115-126. Technomic Publishing Company Inc., Lancaster, PA.

Terpstra, A. H., 2015. The Composition and Production of Fish Feeds, An Overview of Data from the Literature and the Internet. The Netherlands, Anno Domini MMXV.Pdf. Retrieved from https://vdocuments.mx/the-composition-and-production-of-fish-ahm-terpstra-2015-de-alimenti-piscibus.html (accessed 15 January 2021).

Tharanathan, R. N., 1995. Starch: The polysaccharides of high abundance and usefulness. J. Sci. Ind. Res. (India) 54, 452-458. http://ir.cftri.res.in/id/eprint/5486

Van den Einde, R. M., Van der Goot, A. J., Boom, R. M., 2003. Understanding molecular weight reduction of starch during heating-shearing processes. J. Food Eng. 68, 2396-2404.

Williams, M.A., 2000. Interrupted-Flight Expanders-Extruders. Extruders in Food Applications, 63-79.

Yahata, Y., Fukuoka, M., Mihori, T., Watanabe, H., 2006. Water can be made to diffuse against the water content gradient in starchy foods. J. Food Eng. 76, 490-499. https://doi.org/10.1016/j.jfoodeng.2005.05.050

Tables and figures captions

Table 1

Composition of the final extruded pellets (contents of chemicals are expressed in % (w/w) in proportion to the fresh matter).

Table 2

Operating conditions applied to produce the extruded pellets (S1, S2, S3, S4, S5 and S6).

Table 3

Starch gelatinization enthalpy and gelatinization rate for the initial mixture, the preconditioned ones and all the extruded pellets before coating.

Table 4

Twin-screw extrusion parameters, pellets characterizations and pellets usage properties for the S1 to S6 formulations.

Table 5

Distribution in volume of the starchy matrix, the oil-filled pores and the empty ones inside the coated pellets.

Table 6

WSI and WAI results measured on the preconditioned mixture and extruded pellets.

Fig. 1. General view (on the left) and inside (on the right) of a preconditioner (illustrations reproduced with the authorization of the Clextral Company).

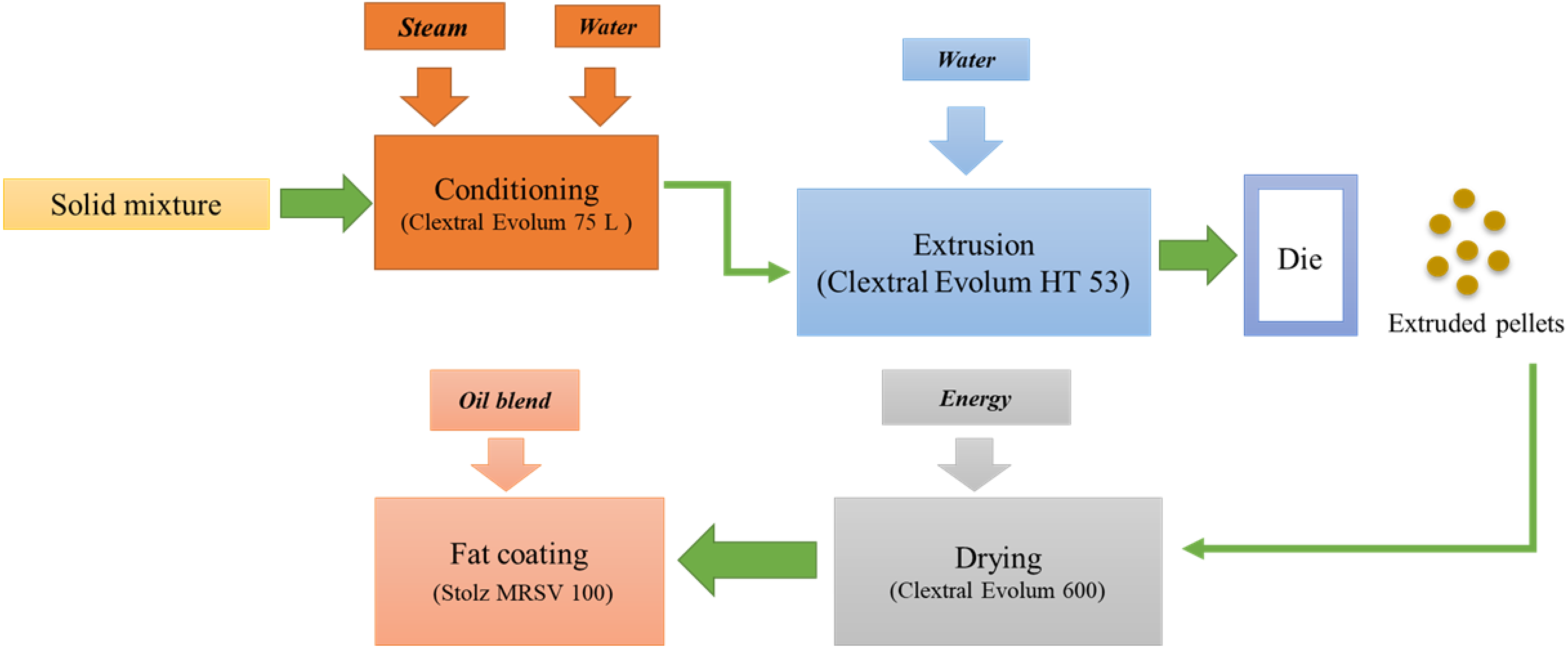
Fig. 2. Simplified diagram of the *Fish Feed* process used to produce the extruded pellets of formulations S1 and S2.

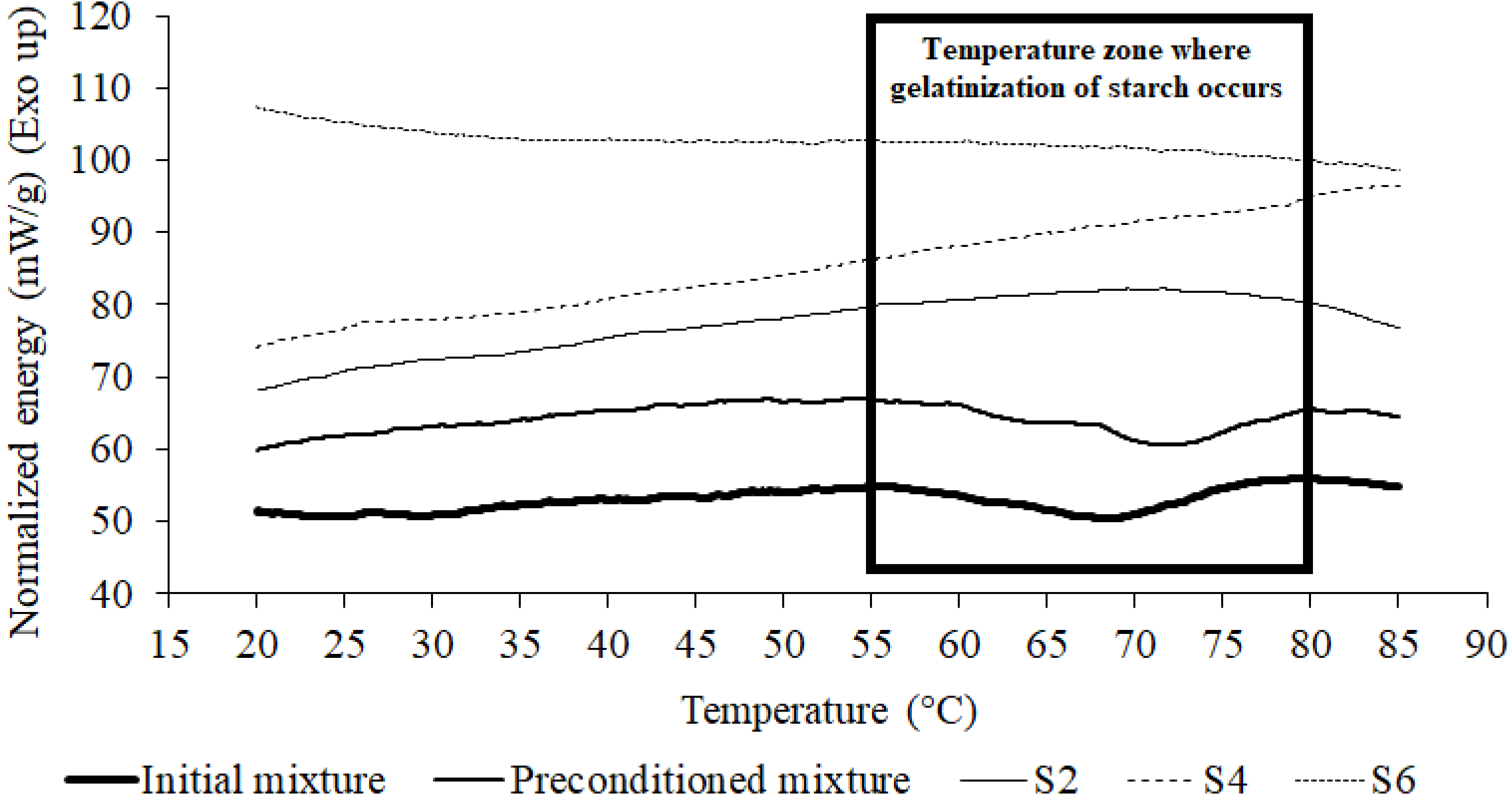
Fig. 3. DSC analysis of the initial mixture, the preconditioned one and the extruded uncoated pellets obtained at 575 rpm.

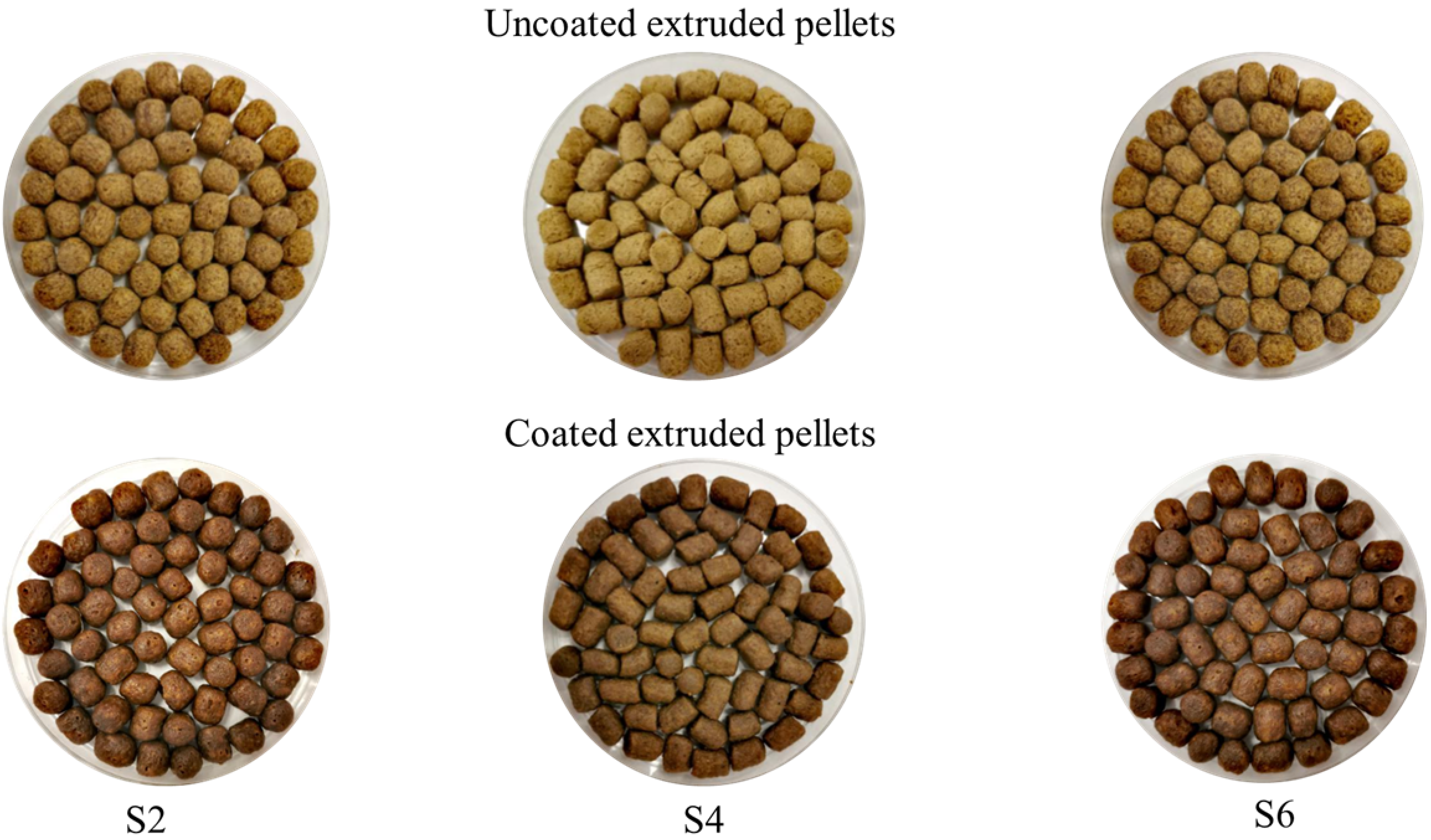
Fig. 4. Photographs of the extruded pellets obtained at a screw speed of 575 rpm (uncoated pellets (dryer outlet) are presented at the top and the coated ones at the bottom; from left to right: S2, S4 and S6) (8.5 cm for the Petri dish diameter).

Fig. 5. Tomography images of *Fish Feed* pellets from formulations (a) S1, (b) S2, (c) S3, (d) S4, (e) S5, and (f) S6 (425 rpm for the screw speed for the three images on the top, and 575 rpm for the three ones below). The volume occupied by the starchy matrix appears in light grey and white, and the empty pores in black. The oil is also visible inside the coated pellets, appearing in dark grey (*i.e.*, slightly darker than the starchy matrix).


Water inlet
Steam inlet
© Clextral
© Clextral


Steam
Water
Solid mixture
Conditioning
(Clextral Evolum 75 L)
Water
Extrusion
(Clextral Evolum HT 53)
Die
Extruded pellets
Oil blend
Energy
Fat coating
(Stolz MRSV 100)
Drying
(Clextral Evolum 600)


Temperature zone where gelatinization of starch occurs
Normalized energy (mW/g) (Exo up)
120
110
100
90
80
70
60
50
40
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
Temperature (°C)
Initial mixture
Preconditioned mixture
S2
S4
S6


Uncoated extruded pellets
Coated extruded pellets
S2
S4
S6

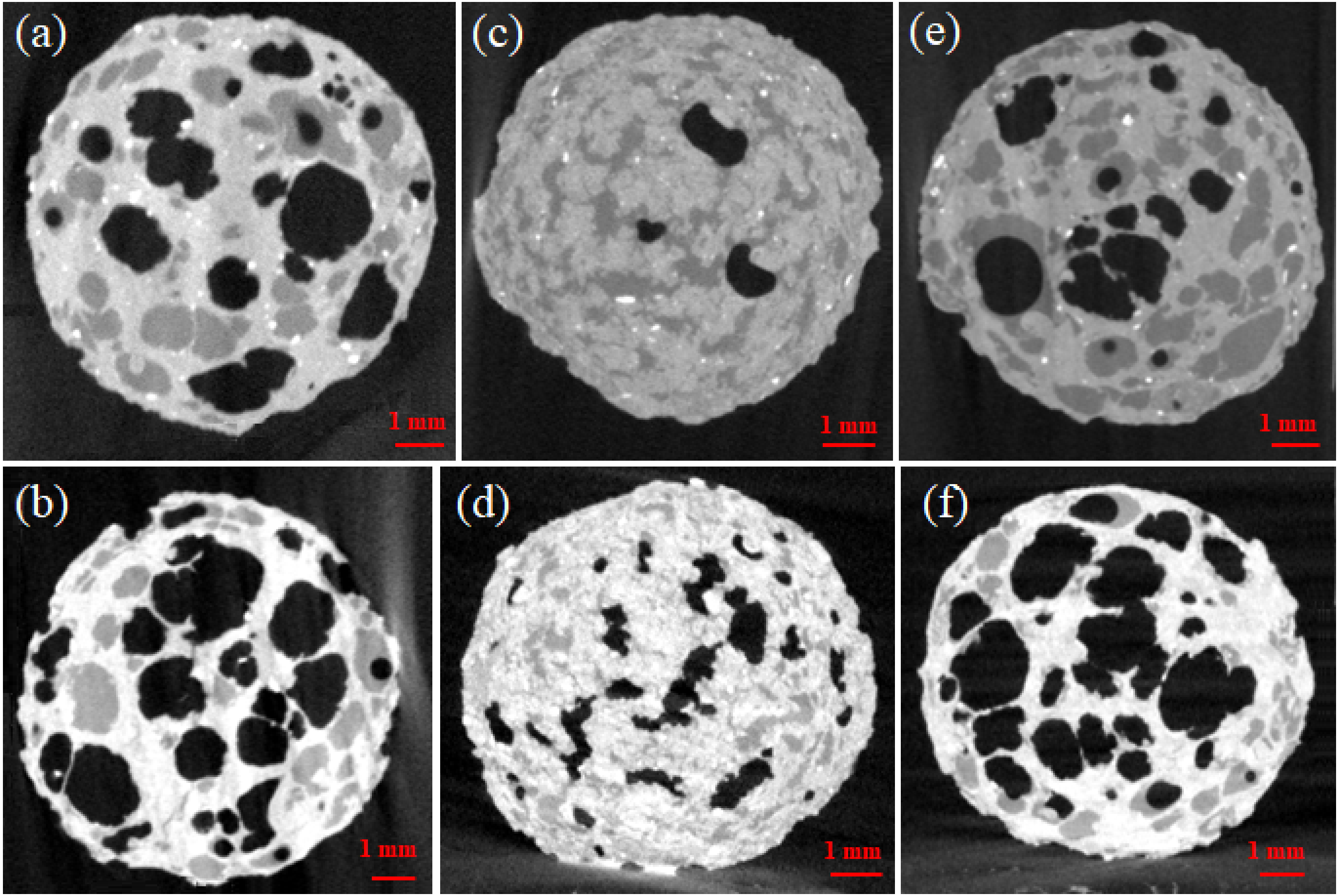

(a)
1 mm
(c)
1 mm
(e)
1 mm
(b)
1 mm
(d)
1 mm
(f)
1 mm

Table 1

Composition of the final extruded pellets (contents of chemicals are expressed in % (w/w) in proportion to the fresh matter).

Chemical	Content (% (w/w))
Crude protein	35.5
Fat	30.0
Starch	11.1
Crude cellulose	2.9
Ash	4.9
Moisture	7.0

Table 2

Operating conditions applied to produce the extruded pellets (S1, S2, S3, S4, S5 and S6).

Operating conditions	**With preconditioner**		**Without preconditioner**			
	S1	S2	S3	S4	S5	S6
Solid inlet flow rate (kg/h)	110	110	110	110	110	110
Screw speed (rpm)	425	575	425	575	425	575
Device's filling coefficient (kg/h rpm)	0.258	0.191	0.258	0.191	0.258	0.191
Q_V (kg/h)	13.2	13.2	0.0	0.0	0.0	0.0
Q_{PREC} (kg/h)	10.9	10.9	0.0	0.0	0.0	0.0
Q_{TSE} (kg/h)	15.6	15.6	26.5	26.5	30.0	30.0
Water-to-solid ratio	0.361	0.361	0.241	0.241	0.273	0.273

The device's filling coefficient is defined as the ratio of the solid inlet flow rate to the screw speed. Q_V is the steam flow rate in the preconditioner; Q_{PREC} is the water flow rate in the preconditioner; Q_{TSE} is the water flow rate in the twin-screw extruder.

Table 3

Starch gelatinization enthalpy and gelatinization rate for the initial mixture, the preconditioned ones and all the extruded pellets before coating.

Code	**S0**	**S1 PREC**	**S2 PREC**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
Material	Initial mixture	Preconditioned mixtures		Powder from extruded pellets (dryer outlet)					
ΔH (J/g)	1.14 ± 0.03	0.45 ± 0.01	0.47 ± 0.01	0.00	0.00	0.00	0.00	0.00	0.00
GR (%)	n.a*	60.5	58.8	100.0	100.0	100.0	100.0	100.0	100.0

** n.a: not applicable; GR: gelatinization rate (%).*

Table 4

Twin-screw extrusion parameters, pellets characterizations and pellets usage properties for the S1 to S6 formulations.

Formulations	**With preconditioner**		**Without preconditioner**			
	S1	**S2**	**S3**	**S4**	**S5**	**S6**
Screw speed (rpm)	**425**	**575**	**425**	**575**	**425**	**575**
Twin-screw extrusion parameters						
Moisture at preconditioner outlet (%)	18.9^{a} ± 0.1	19.0^{a} ± 0.2	n.a	n.a	n.a	n.a
Material temperature at preconditioner outlet (°C)	76^{a} ± 1	73^{b} ± 2	n.a	n.a	n.a	n.a
I (A)	39^{d} ± 2	39^{d} ± 1	50^{a} ± 1	44^{b} ± 1	40^{c}± 1	37^{e} ± 1
SME (W h/kg)	76^{e} ± 3	102^{b} ± 3	97^{c} ± 2	117^{a} ± 4	78^{d} ± 3	98^{c} ± 4
Counter-pressure (bar)	14^{e} ± 3	9^{f} ± 3	35^{a} ± 1	29^{b} ± 2	23^{c} ± 4	19^{d} ± 3
Pellets characterizations						
Moisture at extruder outlet (%)	25.4^{a} ± 0.1	24.7^{b} ± 0.1	19.8^{c} ± 0.2	19.8^{c} ± 0.1	24.3^{b} ± 0.3	24.4^{b} ± 0.3
Bulk density (extruder outlet) (g/L)	396^{c} ± 1	361^{e} ± 5	486^{a} ± 8	377^{d} ± 3	434^{b} ± 6	386^{cd} ± 2
Bulk density (coater outlet) (g/L)	589^{c} ± 1	534^{e} ± 2	687^{a} ± 2	596^{c} ± 4	649^{b} ± 2	565^{d} ± 3
Pellet diameter (dryer outlet) (mm)	8.9^{ab} ± 0.3	9.2^{a} ± 0.3	7.8^{c} ± 0.2	8.0^{c} ± 0.3	8.8^{b} ± 0.3	9.0^{ab} ± 0.3
Expansion ratio (%)	51^{ab} ± 5	55^{a} ± 5	32^{c} ± 3	36^{c} ± 5	49^{b} ± 5	52^{ab} ± 5
Pellet's porosity (%)	47^{a} ± 5	49^{a} ± 1	14^{b} ± 1	23^{b} ± 5	43^{a} ± 3	46^{a} ± 4
Pellets usage properties						
OLR (%) at 25 °C	0.66^{d} ± 0.04	0.83^{c} ± 0.01	1.67^{a} ± 0.08	1.04^{b} ± 0.04	0.75^{cd} ± 0.06	0.83^{c} ± 0.09
OLR (%) at 40 °C	1.37^{d} ± 0.01	1.56^{bc} ± 0.07	2.17^{a} ± 0.00	1.48^{cd} ± 0.00	1.52^{bc} ± 0.08	1.64^{b} ± 0.02
Pellet breakage (%)	0.40^{bc} ± 0.10	0.10^{b} ± 0.00	0.55^{ab} ± 0.05	0.60^{a} ± 0.00	0.19^{d} ± 0.06	0.25^{cd} ± 0.10

Dust (%)	$0.10^{a} \pm 0.00$	$0.01^{c} \pm 0.00$	$0.12^{a} \pm 0.02$	$0.07^{b} \pm 0.00$	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$
Durabilty (%)	$78^{cd} \pm 0$	$76^{d} \pm 1$	$81^{ab} \pm 0$	$72^{e} \pm 1$	$84^{a} \pm 1$	$80^{bc} \pm 2$
Hardness (N/mm)	$17^{b} \pm 3$	$13^{d} \pm 2$	$14^{cd} \pm 2$	$10^{e} \pm 2$	$22^{a} \pm 4$	$16^{bc} \pm 3$
Floatability (%)	$65^{c} \pm 2$	$100^{a} \pm 0$	$0^{e} \pm 0$	$25^{d} \pm 3$	$5^{e} \pm 1$	$86^{b} \pm 2$

Means in the same line with the same superscript letter (a-f) are not significantly different at $P < 0.10$.

Table 5

Distribution in volume of the starchy matrix, the oil-filled pores and the empty ones inside the coated pellets.

	S1	**S2**	**S3**	**S4**	**S5**	**S6**
V_{SM} (%)	$53^{b} \pm 5$	$51^{b} \pm 1$	$86^{a} \pm 1$	$77^{a} \pm 5$	$57^{b} \pm 3$	$54^{b} \pm 4$
V_{PO} (%)	$29^{a} \pm 3$	$24^{a} \pm 1$	$11^{bc} \pm 1$	$9^{c} \pm 2$	$29^{a} \pm 8$	$20^{ab} \pm 1$
V_{EP} (%)	$18^{bc} \pm 2$	$25^{ab} \pm 2$	$3^{d} \pm 1$	$14^{c} \pm 4$	$14^{c} \pm 5$	$26^{a} \pm 2$

V_{SM} is the volume occupied by the starchy matrix. V_{PO} is the volume occupied by the oil-filled pores. V_{EP} is the volume occupied by empty pores. Means in the same line with the same superscript letter (a-d) are not significantly different at $P < 0.10$.

Table 6

WSI and WAI results measured on the preconditioned mixture and extruded pellets.

Formulation	WSI	WAI
Preconditioned mixture	$10.9^{a} \pm 0.9$	$200^{c} \pm 5$
S1	$12.3^{a} \pm 1.1$	$259^{ab} \pm 6$
S2	$12.7^{a} \pm 0.2$	$253^{ab} \pm 1$
S3	$12.7^{a} \pm 1.0$	$242^{b} \pm 2$
S4	$12.3^{a} \pm 0.8$	$243^{b} \pm 3$
S5	$11.6^{a} \pm 0.6$	$274^{a} \pm 12$
S6	$11.7^{a} \pm 0.7$	$277^{a} \pm 17$

Means in the same column with the same superscript letter (a-c) are not significantly different at $P < 0.10$.