# Effects of enzymes and lignosulfonate addition on tensile strength, surface hydration properties and underwater swelling rate of microalgae pellets

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ABSTRACT

This article shows how novel additives affect the physical strength and hydration properties of microalgae pellets made for benthic crustaceans. The article includes novel measurement techniques based on image analysis. These techniques represent a step forward to characterize the shelf life of these pellets during storage and usage phase underwater.

## INTRODUCTION

The need for novel farmed species of biomass, to be used as feed ingredients for aquaculture, have brought large attention during recent decades. Microalgae is a sustainable source of fat and protein, it is less complicated to process microalgae than other vegetable ingredients due to small particle size and lack of lignocellulose. On the other hand, novel additives like enzymes have demonstrated to improve the nutritional utilization of feed ingredients for various aquatic species. However, published data on how the enzymes affects the physical characteristics of aquatic feeds is scarce.

The rheological characteristics of feed pellets is important for trading, storage, transport, animal consumption and as a quality parameter. The strength of pellets, its ability to remain unbroken in the trading line, is one of the most common and demanded quality parameter for trading. Another rheological characteristic, important for aquatic feed, is the deformation of the pellets underwater, which is critical when feeding benthic organisms as it can indicate how long a pellet can remain useful underwater. Pellets that remains cohesive are more likely to be eaten than disintegrated pellets. Pellet disintegration should also be avoided as they disperse nutrients into the aquatic environment. The ratio of deformation of a pellet underwater (e.g. swelling), before disintegration takes place can be an important quality parameter to be brought to the aquaculture industry. This article presents a new method that estimates the rate of swelling of pellets under stagnant water, using a special testing arrangement coupled with image analysis. Another important parameter for shelf life, still not used by the aquaculture industry, is to estimate the ability of a pellet to absorb water and to quantify how hydrophobic or hydrophilic a pellet surface is. For this purpose, an optical tensiometer is used in the article. Water activity is also a parameter described in literature as having great importance for feed shelf life, but not often measured. The effects of enzymes and lignosulfonate (LS) over microalgae pellets is studied in this article and presented with the novel methods.

## MATERIALS AND METHODS

### Raw Materials

The microalgae used for pelleting was produced by Cellana LLC, National Energy Laboratory Hawaii. The microalgae were *Nanofrustulum sp.* and *Tetraselmis sp.,* they were provided in powder form with a 3.3 % water content and 6.3% for LS.

LignoBond DD (Borregaard – LignoTech, Norway) is a lignin-based additive which was used as a binder of algal biomass during pelleting. The function of LignoBond is to improve pellet durability and reduce fines. It has been also shown that LignoBond decrease power consumption during pelleting, for example for cattle feed[2].

The particle size distribution of microalgae was measured by a Mastersizer 3000 optical unit combined with a Aero S dry dispersion unit (Malvern Instruments, U.K.)

### Mixing and preparations for pelleting

An accurate dispersion of trace quantities of additives within the microalgae (see Table 1) represented with no doubt a challenge.

Table 1. Composition of the mixtures.

| Ingredients | C (%) | C+LS (%) | C+NSP (%) | C+Protease (%) |
|---|---|---|---|---|
| **Microalgae** | 100 | 99.5 | 99.99 | 99.994 |
| **LS** | 0 | 0.5* | 0 | 0 |
| **NSP** | 0 | 0 | 0.01* | 0 |
| **Protease** | 0 | 0 | 0 | 0.006* |

*The percentages used follow the recommendations from the suppliers for the feed industry (dry basis).

Protease and NSP comes in liquid form, so they needed to be sprayed over the mixtures. To mix and spray the additives, an intensive mixing was performed to the ingredients using a high shear mixer having three impellers and a tulip-form chopper (Diosna P1/6, Germany). It was used a mixing speed of 250 rpm and a chopper speed of 500 rpm. The spraying was performed with a spraying lance assembled in the mixer (Düsen-Schlick GmbH, Germany, Model 970). The target moisture content was 7% for all mixtures. Measurements of moisture content of the samples taken from different areas in the mixer were used to assess the dispersion of these additives, assuming a homogeneous suspension.

### Pelleting

Pellets were manufactured using the single pellet press method which has been presented in previous publications[1, 3-7].

To characterize the compressibility of microalgae (i.e. compacting pressure versus density), pellets were made at 7, 11.6, 23.3 and 35 MPa using 81°C of production temperature. The temperature chosen is recommended to avoid salmonella contamination through the feed[8]. Pellet density was calculated based on the defined cylindrical shape of the pellets. Pellet length and diameter were measured with a caliper. The rate of compression was set to 2 mm/min through a rod inserted in a 3.5 mm blanc die. The discharge of the pellet was set to a speed low enough to avoid exceeding the compacting pressure. The total retention time of the materials in the channel were about 9 ± 1 min.

The compressibility plot (Fig. 5) showed that microalgae powder do not change its densities when compressed with pressures over 7 MPa (discussed later). Hence, the compacting pressure 12 MPa was chosen for the rest of the work as it has been observed to be within the range of pressures used in ring die pellet press to produce other feed pellets[7].

For the compressibility plot, the bulk density is included. Bulk density was determined by measuring the mass of a known volume of material that has been loosely poured into a graduated cylinder.

### Measurements of pellet strength

Measurement of strength for each pellet were obtained by measuring the first peak force ($F$) in Newtons during a diametral compression at 1 mm/min.

The maximum tensile stress ($\sigma$) for cylindrical specimens is estimated using Eq. 1[5, 9-11], which is commonly referred to as the "Brazilian" or "indirect" tensile test, as the tensile fracture is produced in a disc-shaped material by compressive loading across the diameter[11].

$$\sigma = \frac{F}{\pi \, r \, L} \tag{1}$$

$r$ and $L$ are the radius (m) and length (m) of the pellets, respectively.

Method to monitor the swelling of a pellet under stagnant water

To monitor the swelling rate of a pellet under stagnant water, a special arrangement was designed and is shown on Fig. 1.

The experimental procedure is done by first adding water at room temperature to a glass container having an inner platform. Once the water is at rest, a pellet is carefully inserted using a tweezer to keep the pellet in place over the platform and to keep the water relatively static. The tweezer, the platform for the pellet and the fitting to the data physics optical tensiometer were all made in a 3D printer for ABS material (Mojo Stratasys). The experimental arrangement shown in Fig. 1 is mounted on an optical tensiometer (OCA 15EC, DataPhysics Instruments GmbH, Germany) as shown on Fig. 2.

The lens from the optical tensiometer has a larger magnification to the one needed to display the entire pellet, for this reason, a special fitting was 3D printed to fit another video microscope with lower magnification (Microviper portable video microscope). The entire experimental arrangement can be seen in Fig. 2.

The analysis of the images (e.g. Fig. 11) were done using Fiji open source image processing software[12]. A good contrast is needed between the pellet and the background light to enable the software to identify the area of the pellets in square pixels. The pellet diameter at time zero were used as a reference for the software to convert pixels into millimetres and thus to obtain an area in square millimetres.

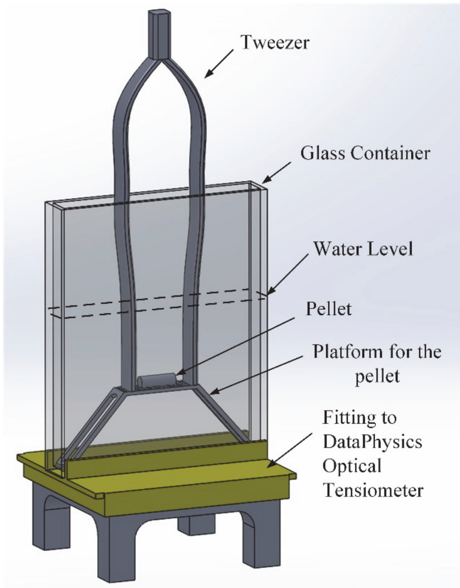


Figure 1. Experimental arrangement for monitoring the swelling of a pellet under stagnant water. The tweezer shown in the figure is used to place the pellet and it is removed during the tests.

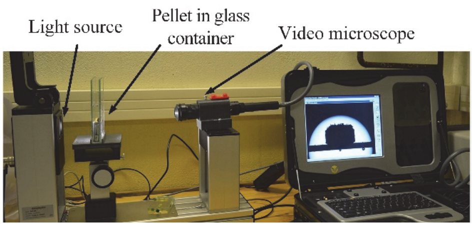


Figure 2. Experimental arrangement to monitor the swelling of pellets assembled in an optical tensiometer

A detailed information of how to utilize Fiji software for this purpose can be found in Catargiu[13].

Method to characterize surface hydrophilicity and hydration properties of pellets

Surface hydrophilicity and hydration properties of microalgae pellets were assessed by measuring the contact angle ($\theta$) of a single sessile water drop placed on the upper plane surface of a pellet (see Fig. 3). If the initial $\theta$ is less than 90°, the surface can be considered as hydrophilic; while $\theta$ greater than 90° indicates hydrophobic surface[1, 14].

Contact angle measurements were conducted at room temperature with a video based optical $\theta$ measuring device OCA 15EC (DataPhysics Instruments GmbH, Germany). A drop of distilled water (2 µl) was disposed from an automatic dosing syringe on the upper plane surface of a pellet and a video of the drop absorption was recorded. Videos of water drop absorption were analyzed by SCA 20 software to measure the initial $\theta$ and its changes with time.

## RESULTS AND DISCUSSIONS

Particle Size Distribution

The analysis of particle size presented on Fig. 4 shows two distinctive peaks for the microalgae (full line) which at first sight could be associated to the two species of microalgae, however it cannot be drawn any conclusion since microalgae came from a milling process after they had been pressed to extract their oil for a biodiesel process. Fig. 4 also shows the particle size distribution for lignosulfonate (dashed line).

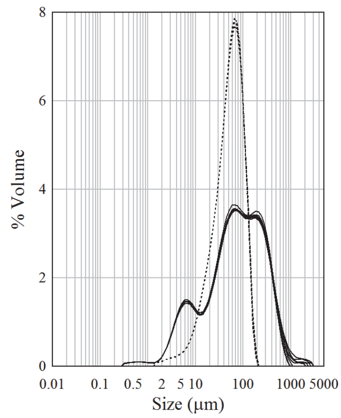


Figure 4. Particle size distribution of microalgae (full line) and lignosulfonate (dashed line).

Pelleting

Regarding the compressibility of microalgae, Fig. 5 shows a sharp increase in density from the bulk values until around 1400 kg/m$^3$ at pressures of 7 MPa. Below 7 MPa, the pellets were not strong enough to keep a cohesive shape during handling. Beyond 7 MPa, it was not seen the further increase of density ($p<0.05$) that it is normally observed in most powders when their volume is reduced during compaction.

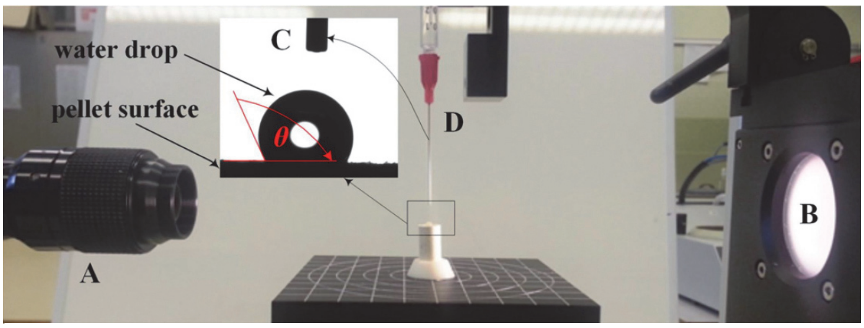


Figure 1. Experimental setup for the contact angle measurements ($\theta$). Letters indicate items as follows: A- camera, B- light source, C- image of a drop on top of a pellet surface for $\theta$ tests, D- dosing syringe with a needle[1]

The reason for this is possibly attributed to an elastic relaxation in the material once the compacting pressure is removed. A similar behaviour has been found previously in pellets made of wheat gluten when pelleting at temperatures and moistures over the glass transition[15].

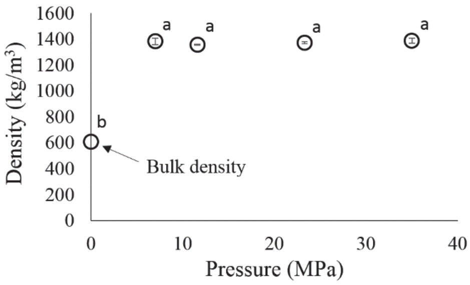


Figure 5. Compressibility of microalgae at different pelleting pressures. Error bars represent the standard deviation. Similar letters indicate no significant differences ($p<0.05$). Averages were calculated from three samples.

Fig. 6 shows a typical stress-strain curve for the microalgae pellets. The sharp reduction in normal force with a clear breaking point indicates the brittle nature of these pellets. The picture of the broken pellets shows that these pellets were broken in tension and thus, it validates the use of Eq. 1 to estimate the tensile strength from the peak force in diametral compression.

As seen from Fig. 7, compacting pressure increased the tensile strength of the pellets ($p<0.05$), even though compacting pressure did not affect the density of the pellets. As it can be seen from Fig. 7, the increase in compacting pressure makes stronger pellets under tensile stresses.

As seen on Fig. 8, protease and NSP decreased ($p<0.05$) the tensile strength of pellets when added in only 0.006% (dry basis) and 0.01% (dry basis). On the contrary, 0.5% addition of LS did not increased significantly the tensile strength.

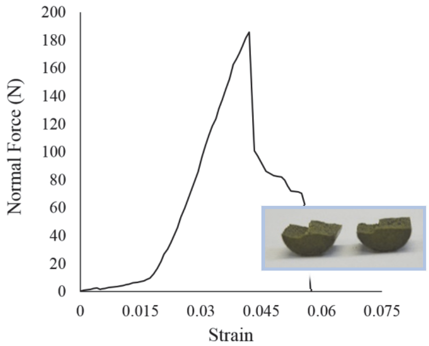


Figure 6. Example of a typical stress-strain curve obtained for the tested specimens. The figure shows at the right bottom a photo of a broken microalgae pellet due to tensile stresses.

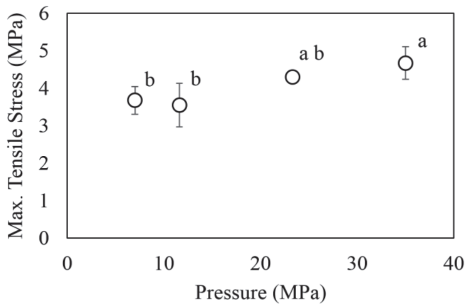


Figure 7. Average tensile strength for pure microalgae at different compacting pressures. Different letters indicate significant differences ($p<0.05$). Averages were obtained from three samples.

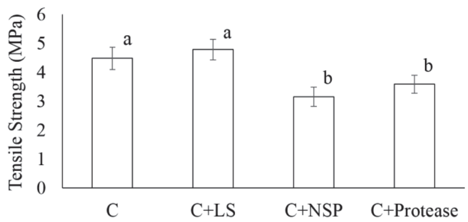


Figure 8. Average tensile strength for the control (C) and when mixed with additives (LS, NSP and protease). Standard deviation is represented by error bars. Averages were obtained from three samples.

The tensile strength of these pellets were similar to pellets made from wheat gluten at 60 °C [15] and larger than pellets made from milk and buttermilk powders[5]. To make a direct comparison with ductile feed pellets tested in diametral compression, where tension is not the main failure factor, one should use Eq. 1 to calculate the maximum peak force for any length and diameter, as it is usually reported for ductile feed pellets.

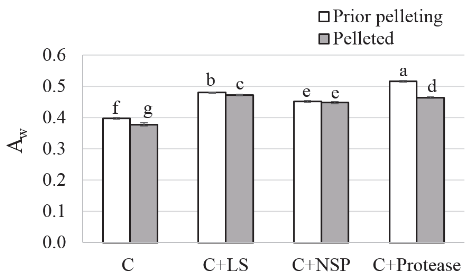


Figure 9. Average water activity ($A_w$) prior and post pelleting for pure microalgae (C) and when blended with LS, NSP and Protease. Different letters indicate significant differences (p<0.05). Standard deviation is represented by error bars. Averages were obtained from three samples

As seen from Fig. 9, pelleting reduced significantly ($p<0.05$) $A_w$ for most of the mixtures and control, except for the mixture C+NSP. On the other hand, all additives increased the $A_w$ for microalgae (C). At first sight, one should analyse the water contents.

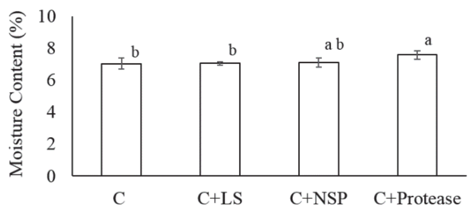


Figure 10. Average moisture content of the mixtures, prior pelleting. Averages were calculated from three samples. Different letters indicate significant differences (p<0.05). Averages were obtained from three samples

According to Fig. 10, the mixtures had a narrow range of moisture contents (~7.0-7.5%), and LS and NSP did not increase significantly ($p<0.05$) the moisture content to the microalgae (C). However, as seen on the next section, LS, NSP and protease decreased the hydrophilicity and thus this could cause the increase in water activity.

<u>Swelling of pellets under stagnant water</u>

Fig. 11 shows an example of how the cross-sectional area of pellets increased under stagnant water. This figure is an example of the type of images that were used to calculate the surface area in the software.

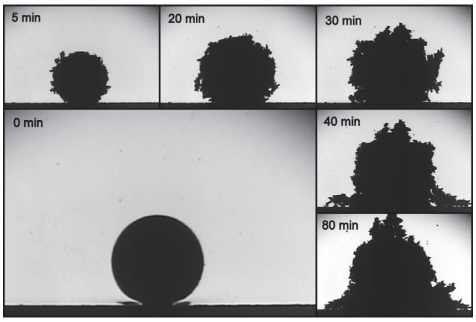


Figure 11. Sequence of pictures in backlight of a pellet under stagnant water from 0 to 80 minutes

Fig. 12 shows how the pellets were swollen, but still remaining cohesive along the testing time.

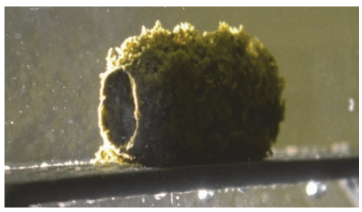

Figure 12. Swollen pellet under water

From Fig. 13 is possible to observe that pellet made of pure microalgae (C) had the largest swelling, this means that all additives reduced the swelling. Protease was the additive that most reduced the swelling and thus it would probably extend the use of the pellets underwater.

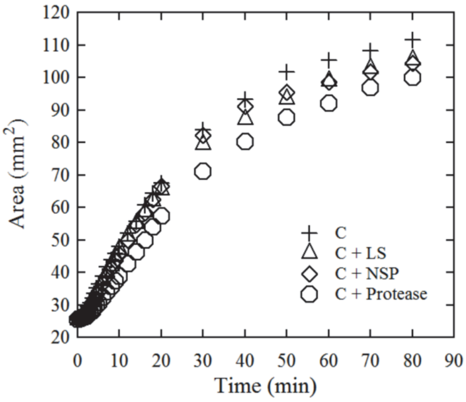


Figure 13. Average cross-sectional area of pellets placed underwater along the testing time.

<u>Surface hydrophilicity and hydration properties of pellets</u>

Fig. 14 shows the typical images of the sessile drop sitting over the pellets observed for the different mixtures at different time intervals.

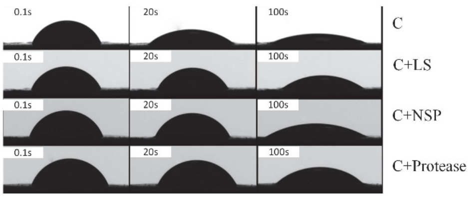


Figure 14. Example of sessile drop at the pellet surface during different time intervals.

According to a Fisher test for the initial contact angle, Protease produced a significant ($p<0.05$) decrease in hydrophilicity when added to the microalgae previous pelleting. However NSP and LS did not decrease the levels significantly ($p>0.05$). Yet, from Fig. 15 it can be seen that along the testing time, the pelleted mixture protease in microalgae presented the highest contact angle (i.e. less hydrophilic), followed in order by the pelleted mixture NSP and LS in microalgae. The lowest contact angle (i.e. more hydrophilic) during the testing period was for the pellets made of pure microalgae (C).

The pellet hydration rate can also be observed from Fig. 15. The control pellets presented the quickest absorption of the sessile water drop followed in order by the mixtures with NSP and LS. The pellets made with protease had the slowest absorption of the water drop.

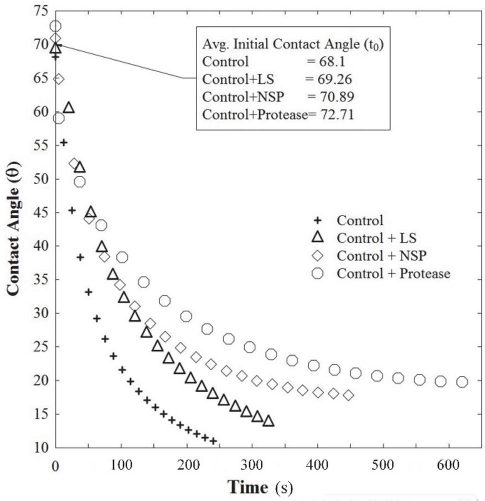


Figure 15. Average contact angles of a sessile water drop placed at the surface of a pellet. Contact angles were measured until total absorption of the water drop.

## CONCLUSIONS

Protease and NSP decreased the tensile strength of pellets when added in only 0.006% and 0.01% respectively, however LS did not change the tensile strength when added in 0.5%. Protease, NSP and LS increased water activity, decreased hydrophilicity, the swelling of pellets underwater, and the absorption rate of a sessile water drop sitting at the pellet surface. Protease produced the lowest swelling and the less hydrophilic pellets followed by LS and NSP.

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