Norwegian University of Life Sciences
Faculty of Science and Technology

Philosophiae Doctor (PhD)
Thesis 2023:69

Towards a sustainable feed pelleting with novel raw materials and enzymes to meet the challenges of developing feed manufacturing

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Acknowledgments

This thesis is the result of 7 years of work that has been done almost entirely in my free time. The serious hobby work has been done in addition to my daily activities as the head of the Center for Feed Technology and lecturer at the Faculty of Biosciences, Department of Animal and Aquacultural Sciences at the Norwegian University of Life Sciences (NMBU).

First and foremost, I want to thank my supervisor, Odd Ivar Lekang for allowing me to perform this work and bear with me for such a long time. Special gratitude goes to my dear old friend and co-supervisor Carlos Salas-Bringas for enormous teaching and contribution in scientific and technical areas. I also owe a debt of gratitude to my dear friends and life coaches Birger Svihus and Ismet Nikqi for making me understand simultaneously the flow of science, the flow of friendship, and nevertheless the flow of life, since the year 2000. Such an honor!

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My ultimate thought after this journey: *Experimenting is a multi-coated understanding of reality in a distorted world without promises to be made.*

"I do not understand the subject any better, but I am now confused at a much higher level!"
Enrico Fermi

Oslo, August 2023 Dejan Miladinovic

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1 Abbreviations, definitions, and symbols

LS – lignosulphonate

NSP – enzymes for non-starch polysaccharides

a_w – water activity

UPS - underwater pellet swelling

p_{max} - pressure at the incipient flow

θ – contact angle

2 Preface and a list of publications

The Doctoral (Ph.D.) thesis was submitted to the Faculty of Science and Technology (Realtek), Department of Process Engineering at Norwegian University of Life Sciences (NMBU), Ås, Norway. The Ph.D. project was financially supported by the Center for Feed Technology (NMBU) and Realtek (NMBU). The Ph.D. thesis is based on a compendium of four scientific papers listed below. The thesis contains the introduction, which reviews and assembles the objectives, theoretical background knowledge, methodology, results, and conclusions of the accompanying papers.

Paper I

van der Poel, A.F.B., Abdollahi, M.R., Cheng, H., Colovic, R., den Hartog, L.A., Miladinovic, D., Page, G., Sijssens, K., Smillie, J.F., Thomas, M., Wang, W., Yu, P., Hendriks, W.H., 2020. Review article: Future directions of animal feed technology research to meet the challenges of a changing world. Animal Feed Science and Technology, vol. *270,* pp. *1-14,* https://doi.org/10.1016/j.anifeedsci.2020.114692.

Paper II

***Salas-Bringas, C.**, Catargiu, A.M., ***Miladinovic, D**., Schüller, R.B., Mišljenović, N., 2015. Effects of enzymes and lignosulfonate addition on tensile strength, surface hydration properties and underwater swelling rate of microalgae pellets. ANNUAL TRANSACTIONS OF THE NORDIC RHEOLOGY SOCIETY, VOL. 23, pp. 153-160.

*Authors sharing the first authorship

Paper III

Miladinovic, D.D., Storebakken, T., Lekang, O.I., Salas-Bringas, C., 2021. The effect of feed enzymes phytase, protease and xylanase on pelleting of microalgal biomass. Heliyon CellPress, pp. 1-11; https://doi.org/10.1016/j.heliyon.2021.e08598.

Paper IV

Miladinovic, D.D., Salas-Bringas, C., Mbuto, J.E., Pashupati, S., Lekang, O.I. Fish meal replacement with torula yeast (*Cyberlindnera jadinii*) and enzymatic treatment can affect the flow resistance in the pelleting die and the physical properties of the feed pellets. Manuscript submitted to Animal Feed Science and Technology journal on 27.06.2023

3 Summary

Single-cell organisms have been identified as promising novel feed ingredients for aquatic animals. The knowledge of optimal downstream processes in the form of compaction or pelleting is, however, insufficient and lacking in the literature. The main objective of this thesis work was fulfilling the gaps and contributing to the knowledge of viable raw material usage in the sustainable world of the future. This work is trying to answer what kind of rheological behavior of the single-cell organisms is expected, when compressed and pelleted in the pelleting die is expected when hydrolyzed in an environment with limited water content. The thesis will hopefully serve anyone who is in need to have a clear starting point in their research with pelleting feed for aquatic crustacea with microalgae and yeast.

Paper I reviews the pros and cons of the current situation in commercial animal feed production responsible for more sustainable food production. Animal feed manufacturing is described more as one of the means for overall sustainable human food production step and not as an objective. The review paper reflects on the feed manufacturing processes that affect hygienic stability and nutritive and physical feed quality. The paper tries to find a balance between feed efficiency and animal health where an objective is to improve commercially available low-quality ingredients and novel ingredients. The overall message is to pinpoint the imperative of creating a high-value feed with improved nutrient utilization. Integration of new technologies while processing novel ingredients is of paramount importance for such improvements so that feed manufactured for terrestrial and aquatic animals will not be subjected to volatile markets. It is essential to decrease global poverty and human malnutrition issues by finding and/or developing alternative feed ingredients. Parallel to many other novel ingredients, the single-cell organisms microalgae and yeast are recognized by scientific publications as vital. However, detailed scientific investigations regarding animal health and the nutritional and technical aspect of the feed pellets must approve that single-cell organisms are good alternatives to commercially available feed ingredients. Furthermore, alternative ingredients should preferably not increase the electrical energy consumption when included in the feed.

Subsequently, **Paper II** investigated the effects of enzymes and lignosulfonate (LS) addition on the technical quality of microalgal pellets where de-oiled *Desmodesmus subspicatus* has been a compaction medium. Surface hydration properties and underwater swelling rate of pellets were also examined as a simulation of the pellets being underwater as a feed for benthic aquatic crustaceans. Novel measurement techniques based on image analysis included in this paper represent a step forward for the shelf-life characterization of the feed pellets during storage and when submerged underwater. The enzymes xylanase (NSP) and protease decreased the strength of pellets when added 0.01% and 0.006% respectively. By adding LS in a dosage of 0.5% the tensile strength of microalgal pellets did not change. Added enzymes and LS increased water activity (aw) and decreased hydrophilicity. Adding protease into microalgal biomass created the lowest swelling and fewer hydrophilic pellets, followed by LS and NSP.

Paper III investigated the dose-dependent effect of enzymes beta 1–4, endo-xylanase (Econase XT), an *E. coli* derived phytase (Quantum Blue), and *Fusarium equiseti* protease on underwater pellet swelling (UPS), pressure at incipient flow (p_{max}) in the pelleting die and tensile strength

of pelleted microalgae *Desmodesmus subspicatus*. Adding Econase XT at the recommended level of 0.01% or Quantum Blue at all three levels (0.015%; 0.03% and 0.06%) reduced the p_{max} of microalgal pellets. This may help to lower the usage of electrical energy during pelleting. Adding different dosages of enzymes did not influence the tensile strength of the pellets, nor the surface contact angle between microalgal pellets and water or oil droplets.

Paper IV investigated how the replacement of fishmeal with the yeast *Cyberlindnera jadinii* in feed designed for the whiteleg shrimp (*Litopenaeus vannamei*) and the addition of protease and endo/exo 1,3-β-glucanase can reduce the flow resistance in the pelleting die during pellet discharge. Replacement of the fishmeal with 0%; 2.5%; 5%; 10% and 20% yeast increased flow resistance *(p_{max})* when adding enzymes to the shrimp feed containing 20% yeast. The tensile strength of pellets with 20% yeast was significantly increased in pellets with or without enzyme addition. Pellets with 10%, 20%, and 100% yeast showed to better repel water when compared to pellets with 0% yeast at 94 seconds observation time of contact surface measurement. Pellets treated with enzymes having 10% and 20% yeast showed lipophobic behaviour when analyzed with contact surface measurement. Enzymes showed to decrease underwater pellet swelling of the pellets with 10% and 20% yeast. Longitudinal surface roughness was decreased for the pellets containing yeast and treated with enzymes. The evidence attained by this research may serve as a starting point for feed raw material alteration and process modification prior to commercial production.

3.1 Norsk Sammendrag

Enkeltcelleorganismer har blitt identifisert som lovende nye fôringredienser for akvatiske dyr. Kunnskapen om optimale nedstrømsprosesser i form av kompaktering eller pelletering er imidlertid utilstrekkelig og mangler i litteraturen. Hovedmålet med denne avhandlingen var å fylle gapene og bidra til kunnskapen om bruk av bærekraftige råvarer i fremtidens verden. Dette arbeidet prøver å svare på hvilken type reologisk atferd en kan forvente av enkeltcelleorganismer, når de komprimeres og pelleteres i en pelletiseringsmatrise når enkeltcelleorganismer hydrolyseres i et miljø med begrenset vanninnhold. Avhandlingen vil forhåpentligvis være til hjelp for alle som trenger et tydelig startpunkt i forskningen med pelleting av fôr for akvatiske dyr med mikroalger og gjær.

Artikkel I gjennomgår fordeler og ulemper med den nåværende situasjonen i kommersiell produksjon av dyrefôr som er viktig for mer bærekraftig matproduksjon. Produksjon av dyrefôr beskrives mer som et av midlene for et overordnet bærekraftig matproduksjonssteg og ikke som et mål i seg selv. **Artikkel I** reflekterer over fôrproduksjonsprosesser som påvirker hygienisk stabilitet samt næringsmessig og fysisk fôrkvalitet. Artikkelen prøver å finne en teoretisk balanse mellom fôreffektivitet og dyrehelse med et mål for å forbedre kommersielt tilgjengelige lavkvalitetsfôringredienser og nye fôringredienser. Den overordnede beskjed fra artikkelen er å peke på hvor viktig er å skape et høyverdig fôr med forbedret næringsstoffutnyttelse. Integrering av nye teknologier ved bearbeiding av nye ingredienser er av aller største viktighet for slike forbedringer. På denne måten fôr som er produsert for dyr ikke vil bli påvirket av volatile markeder. Det er essensielt å redusere global fattigdom og problemene med menneskelig underernæring ved å finne og/eller utvikle alternative fôringredienser. Parallelt med mange andre nye ingredienser, enkeltcelleorganismer som mikroalger og gjær blir anerkjent av vitenskapelige publikasjoner som vesentlig viktige. Imidlertid må grundige vitenskapelige undersøkelser angående dyrehelse samt de ernæringsmessige og tekniske aspektene ved fôrpelletsene bekrefte at enkeltcelleorganismer er gode alternativer til kommersielt tilgjengelige fôringredienser. Alternative ingredienser helst bør ikke øke det elektriske energiforbruket når de inkluderes i fôret.

Artikkel II undersøkte effektene av tilsetning av enzymer og lignosulfonat (LS) på teknisk kvalitet av pellets produsert av avoljet biomasse fra mikroalgae *Desmodesmus subspicatus* som et kompakteringsmedium. Overflatehydrerings-egenskaper og undervanns hevelseshastighet av pellets ble også analysert som en simulering av pellets som kan ligge under vann som fôr for bunnlevende akvatiskedyr. Nye måleteknikker basert på bildeanalyse inkludert i denne artikkelen representerer et skritt fremover for karakterisering av fôrpelleters holdbarhet under lagring og da de er nedsenket under vann. Enzymene xylanase (NSP) og protease reduserte styrken (hardhet) til pellets når de ble tilsatt med dose 0.01% og 0.006%, henholdsvis. Ved å tilsette LS i en dose på 0.5% trekkstyrken (hardhet) til mikroalgepelletsene var ikke endret. Tilsatte enzymer og LS økte vannaktiviteten (aw) og reduserte hydrofiliteten. Å tilsette protease i mikroalgebiomasse skapte minst hevelse og færre hydrofile pellets, etterfulgt av LS og NSP.

Artikkel III undersøkte den doseavhengige effekten av enzymene beta 1-4, endo-xylanase (Econase XT), en fytase avledet fra *E. coli* (Quantum Blue) og *Fusarium equiseti* protease på undervanns hevelse av pellets (UPS), trykk ved oppstart av flyt av pellets (p_{max}) i pelletmatrisen og styrken til pelleterte mikroalger, *Desmodesmus subspicatus*. Å tilsette Econase XT i anbefalt nivå på 0.01% eller Quantum Blue i alle tre nivåer (0.015%; 0.03% og 0.06%) reduserte p_{max} for pellets lagt av biomasse fra mikroalge. Dette kan muligst bidra til å redusere bruken av elektrisk energi under pelleting. Å tilsette forskjellige doser enzymer påvirket ikke styrken av pellets, heller ikke kontaktvinkelen mellom mikroalgepellets og vanndråper eller oljedråper.

Artikkel IV undersøkte hvordan erstatning av fiskemel med gjær *Cyberlindnera jadinii* i fôr pellets med tilsetning av protease og endo-/ekso 1,3-β-glukanase kan redusere flytmotstanden i pelleteringsmatrisen under utkast av fôrpellets. Erstatning av fiskemel med 0%; 2.5%; 5%; 10% og 20% gjær økte p_{max} når enzymer ble tilsatt til fôret som inneholdt 20% gjær. Styrken (hardhet) av fôrpellets med innhold av 20% gjær ble betydelig økt med eller uten enzymtilsetning. Pellets med 10%, 20% og 100% gjær viste seg å avvise vann bedre sammenlignet med pellets uten gjær (0% gjær) under observasjon av kontaktflate-måling opp til 94 sekunder. Pellets behandlet med enzymer som inneholdt 10% og 20% gjær viste lipofobisk atferd når de ble analysert med kontaktflate-måling. Enzymene viste seg å redusere vannopptaket til pellets med 10% og 20% gjær når de var under vann. Den lengdegående overflate-ruheten ble redusert for pellets som inneholdt gjær og som ble behandlet med enzymer. Bevisene som ble oppnådd gjennom **Artikkel IV** kan tjene som et utgangspunkt for endringer i fôrråvarene og mulig modifikasjoner av fôrproduksjonsprosessen før kommersiell produksjon skal vurderes.

4 Research objectives

The specific intentions for this scientific work are divided as:

1. Pinpointing current issues in ever-changing animal feed manufacturing and offering solutions for future directions of the technology, methods, and novel feed ingredients for better management in the feed mills (**Paper I**).
2. To examine how microalgae, as one of the easily accessible novel feed ingredients, together with lignosulphonate, could affect the physical strength and hydration of the microalgal pellets, made for benthic crustaceans, by monitoring the pellet swelling with novel measurement techniques based on image analysis **(Paper II)**.
3. To study how different enzymes and their dosage can contribute to lower electrical energy consumption when a novel feed ingredient, based on microalgal biomass, is pelleted by a single die pellet press, and how this can influence the tensile strength of the pellets, underwater pellet swelling, and interaction of the pellet surface with oil and water **(Paper III)**.
4. To investigate the influence of the novel feed ingredient, yeast *Cyberlindnera jadinii* as a replacer of the fishmeal in the feed with or without adding enzymes, on pellatability and physical properties of the pellets **(Paper IV)**.

5 Outline of the Ph.D. thesis

This Ph.D. study is based on three phases presented in Figure 5.1. Each phase is represented with a unique hypothesis and experimental design. The first phase focuses on the overall assessment of current concerns in the feed manufacturing industry. After considering numerous concerns, a final decision has been made regarding technological and technical subjects involving novel feed ingredients. Unicellular organisms' microalgae and yeasts have been chosen as the experimental materials because they do not compete with the human food supply and thus may contribute to sustainability in animal feed manufacturing. The second phase of the presented Ph.D. work was utilizing oil-drained and protein-rich biomass based on microalgae. Chosen microalgal biomass was based on the unicellular organism *Desmodesmus subspicatus* that is well known for its excellent oil production while growing in a high CO_2 environment. This biomass was used as a model to study the rheological properties of the material during pelleting and its influence on the physical quality of compacted biomass after pelleting with techniques and measurements based on image analyses. The second part of phase two was to understand if any of the three chosen enzymes (xylanase, phytase, and protease) and their different dosages can hydrolyze the respective molecules found in microalgal biomass with limited water content. Restricted water content was used to simulate the commercial feed pelleting process. When water is restricted during feed manufacturing this may well induce a change in the rheological and physical quality properties of the feed pellets. Key considerations in both parts of phase two in this Ph.D. work were to investigate the change of pressure at initial flow in the pelleting die, the tensile strength of pelleted material, pellet hydration, and pellet swelling under stagnant water. Pellet swelling was particularly important due to the possibility to use microalgae as a protein-rich ingredient for shrimp feed. The final phase of the Ph.D. work was focused on investigating the pelleting process and physical pellet quality by replacing the fishmeal with different dosages of the yeast *Cyberlindnera jadinii* in commercial feed treated with the enzymes protease and endo/exo 1.3-beta-glucanase.

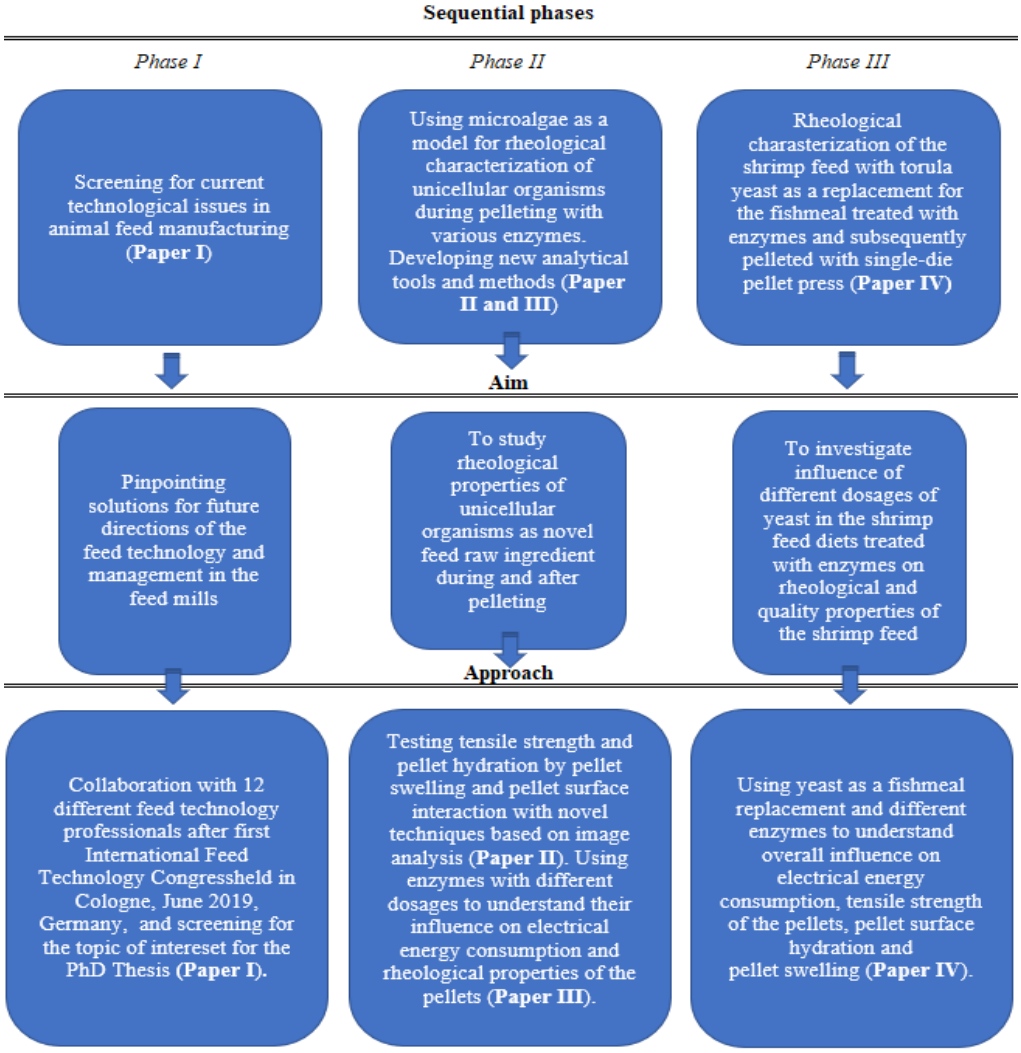

Figure 5.1 Outline of the Ph.D. study.

6 General background

Global growth of the human population is forcing the requirements for alternative protein sources. Concerns over the finite resources of the Earth and the impact of climate change have led to troubling findings regarding the adaptable capabilities of feed manufacturers. These hindrances are based on economic, technological, and marketing deviations and pressures that may influence agricultural outputs in the future (Godfray *et al*., 2010). Sustainable animal and fish feed manufacturing are important for sustainable farming and overall future agriculture. Fish meal (FM) is becoming a progressively luxurious raw material for commercial feed manufacturing. Diversification of expensive protein sources is one of the most crucial aspects of sustainable feed manufacturing and feed formulation flexibility. Thus, there is a large focus in European countries on locally produced and sustainable protein sources identified as by-products, deriving from biorefinery processes. Most of the scientific studies showed that the partial replacement of FM with alternative protein resources can be successfully accomplished with respect to nutritional and health qualities. However, there is limited available data showing how these alternative protein sources can influence the physical characteristics of the feed pellets. The overall challenge for animal and fish farming is to identify economically and sustainably available alternatives to FM and their influence on physical characteristics. General efforts of recognizing, evaluating, and using reasonable feed protein material alternatives are vital for reducing the effect of animal and fish farming on the ecosystem. Impact evaluation of the alternative protein sources must equally involve economic, nutritional, health, and physical characteristics of the animal and fish feed. Physical characteristics can be designed by controlling the rheological properties of the feed particles and their chemistry. Relevant rheological properties can be very broad, thus they may be well understood only through advanced physical concepts. The structure of the feed pellets contains soft and solid matter, for example, amphiphilic molecules, molecular complexes, micelles, colloidal particles, particulate gels, polymer gels, and many others. Therefore, controlling rheology in feeds from a pure experience and without a microstructural perspective should not be encouraged.

6.1 Role of novel ingredients in sustainable feed manufacturing

Diversification of protein sources is perhaps one of the most important aspects related to farmed animal nutrition. Alternative nutrient sources for animal feeds need to be sought and clearly defined as non-human food. Having an accurate nutritional and technical estimation of how novel feed ingredients will result in more effective feed formulations is pivotal for the sustainability process. It is not easy to define biomass as a novel feed material. From a nutritional point, first and foremost it is of paramount importance to understand the chemical composition and the presence or absence of nutritionally active factors. Furthermore, palatability and concentrations of contaminants are important for defining biomass as a novel feed material and as a substitute for commercially available feed materials. From the perspective of the feed manufacturer, criteria such as availability of supply, potential inclusion rates, stability, storage, and effect on the physical pellet quality of the final feed are vital. Moreover, novel feed ingredients should preferably not increase energy consumption during

feed manufacturing, unless inclusion reduces the feed costs. Though usage of novel feed ingredients must have low prices, secure sourcing, and should be environmentally friendly and sustainable. More knowledge regarding the effects of novel feed ingredient processing on technical and nutritional feed quality should be obtained in relation to the impacts of process equipment or process conditions on final nutrient utilization. Novel ingredients should not have a negative effect on the optimal nutritional and physical characteristics of the feed. And finally, the novel feed ingredients should not cause higher feed manufacturing costs.

Unicellular organisms showed to be a good substitute for the commercial protein supply in human and animal nutrition (Bratosin *et al.* 2021). Therefore, such protein-based raw ingredients will considerably be used more for feed manufacturing in the future. Scientific discoveries suggest that unicellular organisms when properly processed, may improve animal and fish nutrition (Jones *et al.* 2020). The inclusion of single-cell organisms as the feed raw materials showed promising scientific results when replacing the fishmeal. Yeasts (Grammes *et al.,* 2013; Vidakovic *et al.,* 2016, 2019) and microalgae (Grammes *et al.,* 2013; Taelman *et al.,* 2013) are two leading and most researched unicellular novel feed ingredients (Ritala *et al.* 2017). Yeasts and microalgae are evolving as sustainable feed raw material alternatives (Øverland *et al.* 2013; Matassa *et al.* 2016). This is partly due to their independence from fertile land and fresh water.

6.2 Microalgae as novel feed ingredients

Microalgae can be used in animal feed products (Dineshbabu *et al.* 2019). They possess useful added value in the form of immune and health-stimulating benefits for aquatic organisms (Grammes *et al*., 2013; Yaakob *et al.* 2014; Muhammad *et al.* 2020). Nutritive values and techno-functional components of microalgae are advocating microalgae as a superior alternative feedstock (Nagarajan *et al.* 2021). Microalgae have high levels of protein, carbohydrates, lipids, and antioxidants, and as such stand out as a promising novel ingredient (Tueling, 2018). Microalgae may reduce the ecological impact of current fish meal usage for feed manufacturing. This is all making microalgal biomass used in aquaculture feed manufacturing an environmentally sustainable option (Taelman *et al.,* 2013). *Chlorella vulgaris* and blue-green microalgae cyanobacteria spirulina (*A. platensis*) are well known for their high protein content. Furthermore, the microalgae *Scenedesmus sp.* and *Desmodesmus s.* are known for their large lipid storage (Xin *et al.*, 2010). *Desmodesmus s.* can be a suitable protein-rich by-product after oil draining as a replacement for the FM (Viswanath, *et al.* 2016).

6.3 Yeast as a novel feed ingredient

Most scientific discoveries show that yeasts as eukaryote single-cell organisms are having an important role as sustainable feed ingredients. The entirety of biotechnology can benefit from approximately 80 species of yeast, adding significant value (Türker, 2014). This is due to their ability, together with enzymatic technology, to transform non-food biomass from the agricultural industry and partially forestry into valuable feed ingredients (Curto and Tripodo, 2001; Adoki, 2008; Mondal *et al.* 2012; Rajoka *et al.*, 2012, Øverland and Skrede, 2017). Having developed quality feed protein ingredients without being dependent on ever-changing

climate, arable land, and available clan water, truly put yeast as a good feed protein alternative (Couture *et al.* 2019; Lapena *et al.* 2020a; Lapena *et al.* 2020b).

The usage of yeast as a protein source for aquatic diets was investigated for over four decades (Mahnken *et al.* 1980) and in different aquatic animal species (Øverland *et al*., 2013; Vidakovic *et al.,* 2016, 2019, Agboola *et al.,* 2022). Compared to FM, all investigated yeast species (*Saccharomyces cerevisiae, Cyberlindnera jadinii, Kluyveromyces marxianus, Blastobotrys adeninivorans* and *Wickerhamomyces anomalus*) have beneficial amino acid profiles, except for methionine, lysine and arginine (Agboola *et al.,* 2020). These amino acids are usually limiting essential amino acids for aquatic animals. Conclusive scientific results show that using the yeast species *Cyberlindnera jadinii* as a substitute for the fishmeal, when fed to fish, may contribute to similar or better protein and amino acid digestibility. Yeast, however, did not change feed conversion, specific growth rate, and final growth rate, compared to fishmeal diets (Øverland *et al*., 2013). The latter authors concluded that the aquatic animals fed with a diet containing *Cyberlindnera jadinii* had increased nitrogen retention, compared with those fed the fish meal diet or diets with other yeast types. Major scientific findings place the yeast *Cyberlindnera jadinii* in the pivotal position when deciding to use alternative protein sources that can replace FM.

6.4 Feed pelleting process and its importance during feed manufacturing

The global feed processing market is exponentially increasing (Fig. 6.1). According to the report developed by Verified Market Research, manufacturing feed pellets covered the largest market share in 2019. The predicted increase from 2020 to 2027 is about 3.9% (VMR, 2021).

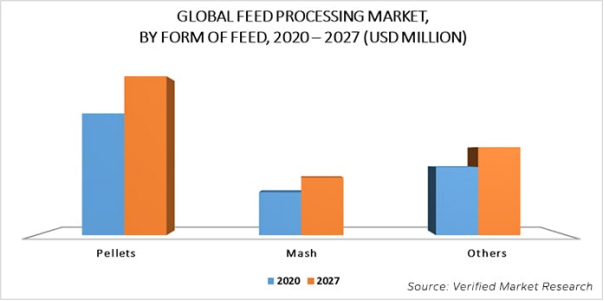

Figure 6.1. - Global feed processing market prediction from 2020 to 2027.

Feed pelleting brings optimal density of the feed with better flow properties during conveying when compared to feed-mash. A variety of feed raw materials are usually mixed to produce animal feed pellets with a nutritive composition designed to meet sanitary, nutritional, and physical qualities. To meet these requirements, it is necessary to understand the various techno-chemical properties of all the ingredients included in the diet. Also, it is essential to know the optimal manufacturing conditions needed for such feed mixtures. Once the knowledge is obtained the feed manufacturing can be optimized and controlled while the nutritional and physical quality of the feed pellets remain maintained. However, nutritional and physical

quality also substantially depends on the downstream processes, for example, cooling, drying, transportation, storage, feeding to animals, etc.

The pelleting process is referring to compressing materials through a pelleting die to create cylindrical feed solids, commonly 2 to 4 times in length size of its diameter. Optimal compressibility can be determined by the rheological properties of the compressed materials that will facilitate the physical (diameter, length, bulk density, surface properties) and mechanical (durability, tensile strength) quality of the pellets. Low physical and mechanical pellet quality is affecting the pellet to break apart during transport, storage, or prior to its usage. Optimal pellet quality keeps the integrity of the compressed feed ingredients when the tensile stress or the shear stress is applied during transportation, storage, or feeding. Feed pellets with good physical quality contribute to high nutritional quality and high feed intake in terrestrial (Skoch *et al*., 1983; Koopmans *et al.* 1989; Abdollahi *et al.* 2013, Suwignyo *et al*. 2022) and aquatic animals (Aas *et al.* 2009; Cai *et al.* 2022).

The optimal physical and mechanical quality of the pelleted feed may be well navigated by minor adjustments in the temperature during steam conditioning. The selection of the die and rollers as well as the gap between rollers and the die affect the pellet quality (Miladinovic and Svihus, 2005; Miladinovic, 2009). However, these adjustments and changes are often subject to the experience and reasoning of the operator. The physical quality of the final feed product is highly dependent on feed formulation and its rheological properties, the moisture content in the mash, temperature of the feed mash during the pelleting and cooling procedure. The overall idea of modern feed production is to come up with a toolkit that will support decisions for process optimization and selection of the feed raw materials. Such a toolkit will enhance the opportunity of the feed manufacturer to achieve the best possible physical and mechanical quality of the feed pellets with limited use of energy for the provided feed formulation.

Measurement of rheological behavior in the pellet press of a single raw ingredient used for animal feed manufacturing is very challenging. However, if achievable, such information can provide significant evidence for predicting the behavior of the feed-mash diet during the manufacturing process. By understanding the rheological properties of a single material, one may assess the structure and technical quality of the final feed product which is manufactured as a mixture with other materials compacted together. Along these lines, the end-use properties of the feed pellets can be assessed and modified. General challenges and nonetheless concerns in the feed technology are based on the physicochemical properties of the raw ingredients, principally affected by the climatic origin and processing history. Figure 6.2 summarizes general concerns and challenges frequently encountered during feed manufacturing.

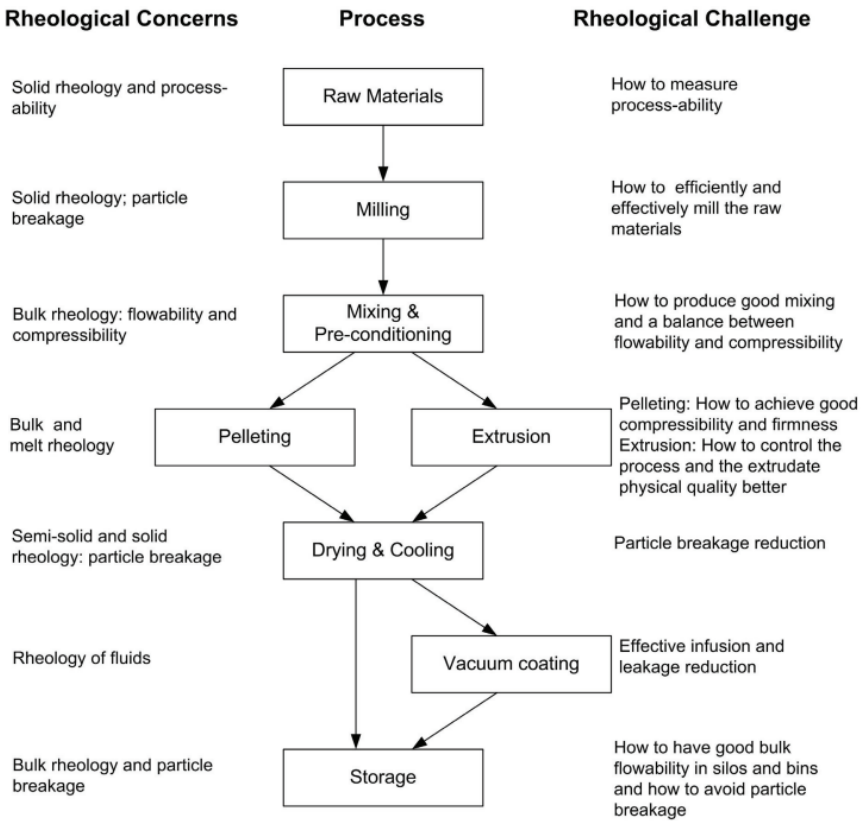

Figure 6.2. Block diagram summarizing the main rheological concerns and challenges within feed manufacturing (Source: Salas-Bringas *et al.* 2008).

6.5 Densification (interaction between particles) and compaction (interaction between particles and compaction die-wall) during feed pelleting

The feed pelleting process includes both densification and compaction of mixed feed ingredients. A process that purposely combines small particles into larger ones, where the original components cannot be identified can be defined as size enlargement. Enlargement of feed material powder particles is used to control flow and reduce dust formation during transportation. Agglomeration and pelletizing are all processes that include compaction and binding of powder materials. The objective of such processes is to control the density or porosity of the final product. To understand and predict the quality of the feed products based on powder constituents it is of paramount importance to understand the behavior between feed ingredients during densification and compaction. Modern processing of the feed ingredients carries an effort to alter the active molecules from powder particles into the final feed products. These products should have assumed microstructure that could improve the physical, nutritional, chemical, and other desirable properties of the final products. Each feed raw

material in a heterogeneous and homogeneous mixture has its properties that may influence the densification and compaction of particles that can change the final properties of the product.

The mechanism of bonding within the feed mixtures is based on the adherence of powder particles to each other to form a larger system with a structure that is supposed to tolerate deterioration. Particle size and its distribution within the feed mixtures, together with moisture content and surface tension of the bonding medium could be identified as factors influencing the agglomerated or compacted systems, the feed pellets.

6.6 Physical chemistry of feed mixture powders

All particles in animal feed, their molecules, ions, and atoms are subjected to forces between them, with or without applied stress to them. These interaction forces may cause some chemical reactions between particles. Between the components of the feed mixtures, there are various types of forces described as bonds. The most important ones are explained by Walstra (2003):

a. Hard-core forces with very large energy where the direction is not fixed. These forces cannot lead to bond formation due to its repulsive behavior. These forces between powder particles are rare in practice;
b. Covalent bonds involve medium energy with a very restricted range of directions and restricted attraction properties. Covalent bonds may, however, be very strong;
c. van der Waals forces involve very low energy. Though, they have a wide working range and are without a fixed direction. These forces act between all molecules and with different interactions where the positive end of one molecule orients to the negative end of another molecule. The structure of feed powders is mostly determined by van der Waals forces between small particles (<100 m^1). Because of such low energy forces, even when additional stress between particles is applied, the end structures are loosely packed. This could lead to process-related issues and low physical quality feed products;
d. Hydrogen bonds are also based on low energy. These bonds are having fixed direction. All hydrogen bonds are having attractional properties between a covalently bound hydrogen atom and one electronegative group, like oxygen or nitrogen. Hydrogen bonds are weaker than covalent bonds but much stronger than van der Waals interactions.

These bonds and their interactions may influence the frictional forces acting between molecules of the feed particles. Thus, they can influence the compaction flow of the material or the interaction between the particles and their active molecules during densification.

Chemical interactions between molecules of the particles at elevated temperatures during densification and compaction can influence the viscosity of the feed mixture. Thus, the rheological properties of the feed mixture may change due to convection, heat conduction, and diffusion of the single raw material included in the feed mixture. Such changes might further influence compacting properties of the entire system.

Mechanisms responsible for inter-particle reactions and final stability of compacted powder systems are well defined by Pietsch (1991) as solid bridges, immovable or mobile liquid bridges, attraction forces between particles and interlocking bonds.

Solid bridges are created between clustered particles as deposited material developed by diffusing molecules from one particle to another. This can happen through incomplete melting at contacted sites of the particle where elevated temperatures and pressures progress. Also, the solid bridges might be developed by chemical reactions everywhere where the hardening of added binders or solidification of molten feed components would occur. The force influencing the cohesion of powder particles will thus rest on the contact area of the solid bridges and the diameter of the particles. From another point, forces influencing bond-creation between particles that come from the surface tension of liquid structures and capillary pressures that are formed during compaction are defined as liquid bridges. Very viscous feed mixtures could use mobile liquid bridges to create immovable liquid layers that can decrease physical distances between particles and hence increase contacts between them. Immovable liquid bridges created by constrained liquid molecules could produce very strong bonds. The capillary pressure and interfacial forces can also create strong bonds between feed particles by mobile liquid bridges. However, these bridges may be weaker than immovable liquid bridges. Attraction forces between particles are purely dependent on surface properties formed between the particles, strongly defined by van der Waals forces. Interlocking bonds are pure mechanical bonds between particles. These bonds are weak when compared to previously mentioned mechanisms, however, they are very important as reinforcement during establishing the bonds between particles. The point of interaction during particle interlocking is physical contact where the distance is so small that the binding bridges can form several interaction sites on only one particle (Barbarosa-Canovas *et al*., 2005). Interlocking is dependent on the geometry of particles, porosity, and coordination number as a valuable microscopic parameter that describes the packing of the particles.

6.7 Flow and deformation characterization of feed mixture powder systems

There are many substances included in the feed mixture powder systems that simultaneously possess both solid and liquid properties when changing the environment. Therefore, the rheology of such systems needs to consider deformation for solid-like components and flow for predominantly fluid-like substances. The flow distance (l), time (s), and mass (m) are important for explaining the rheological properties of the feed mixture powder systems during its flow within the area (A) and the volume (V) when the force (F) is applied.
The unit of F symbolized with the unit newton (N), as a dimension of $m * l * s$, is defined as the load. Load during feed pelleting can be explained as the force to which a feed mixture powder system is exposed in supporting a mass of a powder system by simply resisting externally applied forces in the die hole. Feed mixture powder systems are predominantly densified and compacted in the die hole during feed pelleting. In the compaction process, the stress (σ) is commonly used to explain forces, defined by the unit of Pascals (Pa). These forces influence the feed mixture powder system to be compacted in the pelleting die, where $Pa=N/m^2$ and m^2 is the unit of compaction area. Stress is calculated as presented in eq. 6.1:

$\sigma = F/A$ Eq. 6.1

Stress is achieved when F is applied evenly to the feed mixture powder system enclosed in compaction area (A) within densification volume (V) (m^3), practically called the pelleting die hole. If the feed mixture powder system is theoretically having uniformly shaped particles with similar particle sizes, the deformation of such a system is much lower than the system with various particle size distributions and different shapes of particles. If the geometry of all particles in the system is uniform, it is assumed that stress (σ) is evenly distributed within the entire feed mixture powder system. However, this is very uncommon in feed mixture powder systems due to the un-uniform size, various structures, and chemical composition of the particles representing the mixed feed ingredients. In most powder systems it is often impossible to map the distribution of stress all over the densification volume. Therefore, the rheological characterization of such systems with shear strain (ε) is needed. Shear strain describes the change of the feed mixture powder system when subjected to σ during densification and compaction. Strain during pelleting could be explained by the natural strain as the dimensionless logarithmic ratio of the Hencky strain (eq. 6.2). Hencky strain refers to natural strain as a logarithmic change in size or shape of the material subjected to stress.

$\varepsilon = ln\ (l / l_0)$ Eq. 6.2

where: l represents the stressed height of the powder system and l_0 represents the unstressed height.

Powders have characteristics of a solid during mechanical stress. Also, powders can flow, which is characteristic of a liquid. All these characteristics are time-dependent. In addition, powder particles can be dispersed in the air, for example during mixing. When powders move within the system because of force imbalance by the gravitational forces their particles show adhesion (molecular interactions) and friction (mechanical interlocking) behavior. The electrostatic force influences the flow of the powder in the compaction systems. Improved flow of the powder systems could be achieved when using larger particle sizes and when particle-to-particle separation is increased (Kendall, 1994). The flow of the feed mixture powder system could be explained as plastic deformation of the system due to the loads acting on it. As explained by Schulze (2008), if the yield strength (f_c) is increased at the surface of the die, that will consequently increase a layer of the powder system. Thus, it will be more difficult to initiate flow through the pelleting die-hole and the pelleting equipment will block. Measuring the flowability of the powder system is possible by measuring the magnitude of the load that is necessary to move that system. Processing conditions prior to feed pelleting could greatly influence the flow behavior of the particles that belong to the feed mixture powder system. However, it must be taken into consideration that the flow property of the feed mixture powder system can be, among other factors, influenced by the moisture of the materials, particle shape, and particle size. Also, the flow property will depend on the chemical nature of the material and its surface roughness before applying any processing conditions. All previously mentioned could be a good reason for challenged or non-successful scale-up processes.

Flow measurement can predict the behavior of the feed mixtures by studying relations between the applied forces (F) in the pelleting die that act on a given feed powder mixture or single raw material. Enough information on the deformation of a single material in the pelleting die may predict the behavior of the feed powder system because of a chemical change due to a change of F or due to the addition of water, steam, or enzymes that may influence that change. Deformation identified as a compaction of the feed mixture powder system during pelleting includes information regarding the applied force and flow distance during that deformation. At this point, the mechanical work could be assumed as a product of those two factors. Therefore, the formula for deformation $W=F\cdot s$ explains that F is a force that influences displacement of the feed material (s) in the direction of F. Displacement of the feed mixture powder system during pelleting, because of densification and compaction of the feed mixture powder system may be explained by laminar flow of the system and its particles. There could be several types of laminar flow during pelleting. The type of laminar flow is dependent on the moisture of the materials, particle shape and size, the chemical nature of the particles in the feed mixture powder system, and the surface roughness of the particles, presented in Figure 6.3.
Each laminar flow is dependent on its geometrical limits defined by the linear flow velocity (v), the velocity gradient (Ψ), the rotation rate (R), and rotation frequency (ω). In high-viscosity feed materials, the ratio of the internal forces to the shearing force of the die-wall can be described with a low Reynolds number (Re) due to slow movement of the feed mixture powder system, and consequently laminar flow of the system through the die.

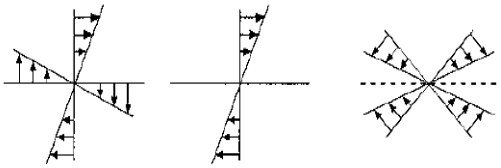

Figure 6.3 – The velocity profiles for different laminar flows, A – Rotation; B – Simple shear; C - Hyperbolic laminar flow. Adopted from Walstra (2003).

Normally during feed pelleting the laminar flow dominates. However, the transitional type of flow can occur too. If the powder system starts rotating in the center due to for example the high content of moisture in the powder or due to any other reason the powder particles will move by rotation (Fig. 6.3.A). Such flow could be defined as simple shear but not as simple as presented in Figure 6.3.B. However, the flow of the feed powder systems in a confined tube (pelleting die hole) during the axisymmetric flow the in the x direction is very unlikely to happen in the pelleting die hole (Fig. 6.3.C). During feed pelleting, the flow is mostly laminar (Fig. 6.4). Theoretically, during such flow, the linear flow velocity is close to zero at the die wall and it is maximal in the center of the pelleting die. The velocity gradient is a shearing stress developed between the layers of different velocities and it equals zero in the center of the die hole. Such shearing stress is maximal at the die-wall. For non-Newtonian systems such as powders, the ability to resist deformation by shear or tensile stress is dependent on the share rate.

The velocity of the feed mixture powder system during compaction in the pelleting die is dependent on the nature of the molecules that create the powder system. During compaction, these molecules experience constant heat motion due to the velocity gradient change and increase of the kinetic energy between particles or between layers of the feed mixture powder and the die wall. However, the pelleting die hole is never long enough for the heat to be fully increased in the center.

Applying constant stress and load to the feed mixture powder system during pelleting makes the system deform. When the deformation of the system under a certain load is high enough the system will start moving. The moment of maximum pressure at incipient flow for the system to deform enough and move forwards is defined as p_{max}. Deformation and dynamics of the system in the pelleting die hole constantly change due to thermo-mechanical change, chemical properties of the compacted feed mixture powder system, the applied stress on the system, etc... In natural polymers, such as proteins or polysaccharides, even a small change in the system, as for example available free-water molecules, may affect the deformation and thus p_{max} of the feed mixture powder system.

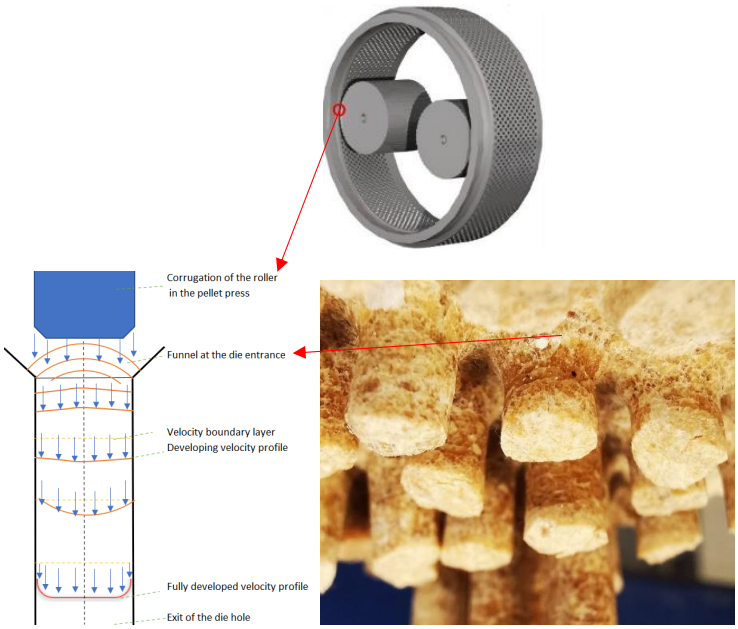

Figure 6.4 - Plug flow with different velocity profiles in the pelleting die hole influenced by centrifugal forces and loads from corrugation of the pelleting ring-die roller. Ring die model: Salas-Bringas (2011).

Some solid materials may move easily through a pelleting die with the low yield stress applied to the materials if it contain enough fat or water. Pressure distribution in the pelleting die is dependent on the shear stress between the compressed feed mixture powder and the die wall, within the length of measured pressure change and the radius of the pelleting die for a given length of pressure measurement. The scale of the material flow through the pelleting die may

depend on the applied yield stress and the observation time. The yield stress is a product of the pressure difference from the measuring points in the pelleting die when a minimum pressure has been obtained and the radius of the pelleting die divided by 2 measuring lengths, one on each side of the die diameter. The viscosity of the particles which are moving in the pelleting die is affected by the applied shear rate. As described by Walstra (2003), all the molecules without perfect spherical shape would affect molecular orientation to be aligned with the direction of flow of the powder system. The plug flow for the compressed feed mixture in the pelleting die is forming when the shear stress is smaller than the yield stress.

After compression of the feed-powder system, a compacted feed pellet enters the phase of relaxation. Such relaxation, better known as the "spring back effect" is explained in Figure 6.5 and it normally arises due to the expansion of residual compressed air and due to elastic recovery of the feed material.

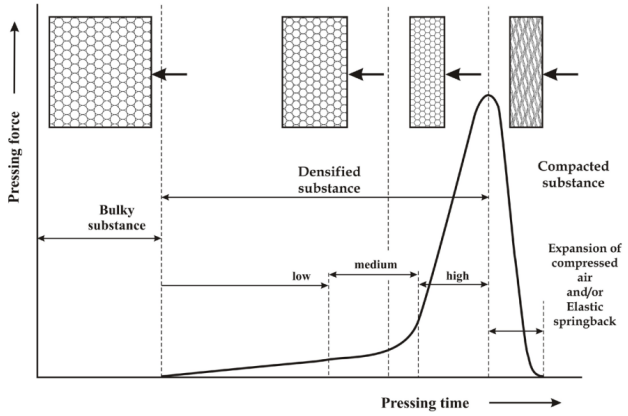

Figure 6.5 – Stages of powder densification and compaction with the elastic spring-back (Pietsch, 2008).

The threshold of the ability for a powder system to flow through the pelleting die and yet to have elastic properties defines the entire system as viscoelastic. Figure 6.6 demonstrates the deformation of a powder system during densification and compaction. When applying load at the feed mixture system that fills the pelleting die hole it can be observed an immediate compression of the system. Such compression is showing instantaneous elastic deformation followed by a prolonged and continuous rate of deformation called "creep deformation". Deformation in densified and compressed powder systems continuously increases with time. When releasing the load on compressed powders there is a sudden partial elastic recovery of it. Such recovery is observed within a certain time, and it is defined as "creep recovery". The creep recovery is usually not high in the feed mixture powder system after pelleting and thus the compacted system does not return to its original height. This is known as permanent deformation, easily shown by size or shape after the pelleting and tableting process.

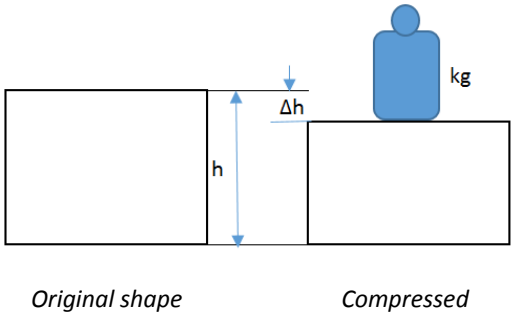

Figure 6.6 - Uniaxial deformation of a solid under a constant force

6.8 Densification and compaction of feed mixture powder systems

During the densification and compaction of the feed particles, there are many variables influencing these activities. Perhaps the most important variables to consider prior to pelleting are the geometry of the particles, their size, and the size distribution of the particles within the feed mixture to be densified and compacted. However, the moisture content of the particles, the thermal property of whether the solitary ingredient which belongs to the feed mixture system or the entire feed mixture powder system, should not be neglected. Also, chemical, and mechanical properties of the materials and their binding characteristics, surface abrasion of the particle, requested final porosity of the manufactured product after compaction, production capacity, total cost, and energy requirements are among many other very important properties to consider prior to manufacturing. Production capacity during pelleting, production costs, and nevertheless density of the feed mixture can be directly influenced by the properties of the die hole. Wall friction in the die hole will depend greatly on previously mentioned factors and properties of the material that the pelleting die is manufactured from. Prior to commencing the feed pelleting process, it is crucial to have knowledge of the feed mixture's density, as it serves as a significant metric. In such a way, the correct production capacity and thus final quality and manufacturing cost can be established. It is important to stress that when the temperature of the compacted feed mixture system elevates the density of such system can change as well, depending on free-water content. For some materials, minor changes in temperature may aid the thermal expansion of the material and thus change all other following processes.

6.9 Influence of the temperature during densification and compaction of the feed mixture powder system

The temperature elevation in the feed pellets after pelleting is a sign of increased kinetic energy of a powder system during compaction (Wang *et al.* 2020). Motion between particles during densification and compaction also increases molecular motion. With increased molecular activity the kinetic energy also increases. Forces applied to the feed mixture powder system during pelleting assist to increase the speed of molecular motion and hence the minor raise of the temperature within the entire system. Thermal energy (E) of densified and compacted feed

mixture is the fundamental kinetic energy of the molecules defined by temperature (T) and measured in the system (eq. 6.3).

$$E = k * T \quad \text{Eq. 6.3}$$

Where: k *is* Boltzman's constant (1.3807×10^{-23} $J*K^{-1}$); $\boldsymbol{T}$ – temperature in Kelvin (K)

Feed mixture powder system with high internal temperatures indicates that molecules of the system have high kinetic energy. Hypothetically speaking, if the feed mixture system is at zero degrees the temperature of the molecules within such mixture will be without any kinetic energy. The internal energy (U) of a thermodynamic powder system may exist in two forms, the heat transfer (Q) and work transfer (W'), and it can be expressed as:

$$U = Q + W` \quad \text{Eq. 6.4}$$

W' is defining the forced displacement of the particles during compaction where pressure and volume can change. Whereas displacement work (W) is a product of pressure (p) and volume (V) of a displaced feed mixture system, defined in equation 6.5.

$$W = p * V \quad \text{Eq. 6.5}$$

The internal energy (U) of the feed mixture powder system during pelleting is a product of the heat transfer (Q) within the system and applied pressure (p) that is influencing the change of the volume in the system. Equation 6.6 explains how the internal energy within the feed mixture system develops during pelleting and equation 6.7 explains the heat transfer within the system.

$$U = Q - p * V \quad \text{Eq. 6.6}$$

$$Q = U + p * V \quad \text{Eq. 6.7}$$

Every powder system despite its chemistry has a thermal property that shows the ability of the material to hold and store heat. Such thermal property, defined as the heat capacity is quantified by specifying the amount of heat needed to raise the temperature to the desired level during manufacturing. The heat capacity of the powder system at constant pressures is defined as:

$$C = Q / T \quad \text{Eq. 6.8}$$

where: C is heat capacity ($J*K^{-1}$); Q is heat (J); T is the temperature (°K)

The heat conductance of the powder system in limited volume during pelleting in the pelleting die is significantly present due to steam addition and friction obtained by particle-to-particle or particle-to-wall interaction. However, some heat transfer resistance is possible to arise within the powder system, and therefore the heat conduction can be minimized. The hypothetical layer in the feed mixture powder system that divides the tempered and non-tempered layers can minimize heat conduction. Such a layer is usually formed in the center of the feed pellet, and it represents the surface heat transfer constant (α). The heat transfer of such layer, with a defined area (A) from the original tempered surface (Ts) to another layer with temperature ($T\infty$) can be expressed as:

$$Q = \alpha * A * (Ts - T\infty) \quad \text{Eq. 6.9}$$

Within the feed mixture powder system, the thermal conductivity is insignificantly dependent on the temperature of the system. This is because the thermal conductivity increases marginally with temperature increase. Insignificant dependence is mostly reliant on water content and the air found between the particles. In a feed mixture powder system intended to be pelleted, there are solid feed particles and water. Here the heat will be transferred by convection when the mass of particles is in motion, and conduction when the electrons move within a particle or when particles collide. The heat transfer is greater if it occurs by conduction. However, this is not the case with dry porous materials, grain, or other powders that are used to create a feed mixture powder system. The heat transfer is happening in this case by convection where the heated air tries to travel through the porous pelleted material. Natural convection of the air always occurs in the direction opposite to gravitational forces. When the air temperature increases, the air travels above the cooler air in the system that is being compacted which has a greater density. That is the reason why the temperature increase in the powder systems during compaction opposite of the gravitational force. In colder climates, such a random heat transfer may cause the blockage of the pelleting die. To overcome this problem, the pellet press should be pre-heated prior to pelleting.

6.10 Compressibility of the feed mixture powder system

Powder in the pelleting die shows an elastic behavior explained by the volume reduction of the powder system when pressure is applied to it. When pressure is removed, some powders may return to their original position but most of the food, feed, and pharmaceutical powders can only insignificantly return to their original position. The mechanical energy applied to the feed mixture powder systems during compression is converted to heat between the feed particles. This mechanical energy is greatly influenced by the unit of stress (Pa), defined by units of force (F) in newtons (N) over the area (A) in square meters (m^{-2}), which is explained by the formula $N * m^{-2} = Pa$. The mechanical energy exchange during compressibility of the feed mixture is happening between the interacting feed particles. Forces acting between the particles and their magnitude and directions must be well comprehended to fully understand densification and

compression during pelleting. When the feed mixture powder system is subjected to stress, the load acting on them will be theoretically transferred through individual particle contacts. Such a powder system is considered as a continuum during compressibility and thus it can be assumed many contacts in a compressing volume (die hole) where the particles are densified. Therefore, it should be assumed that the stress applied to such a system is homogeneously and randomly distributed across the entire densification area. In reality, the particles within the feed mixture powder system are distributed randomly and in three dimensions. Therefore, some particles are in very close contact, whereas other particles are more distant when the stress is being applied.

Normally, during the compression of the particles by pelleting, the entire system of particles is uniaxially compressed in the *x* direction (Fig. 6.7.B). Due to stress caused by compression, the particles approach each other in the *x* direction. The force (*F*) and stress (σx) can be transferred only from one particle to another particle through their physical contact. Due to the deformation of the loaded feed mixture powder system (Fig. 6.7.A) in confined volume (*V*) of the die and in *x* direction, the force chains are oriented in the *x* direction (Fig. 6.7.B). Some particles could have the force chains oriented also in *y* direction (Fig. 6.7.C). Such force could be responsible for the stress (σy) even though the uniaxial compression towards the plane *x* (σx) which is primarily influencing the compressed system as the major stress. Therefore, particles during compaction in *x* direction will be oriented like columns (Fig. 6.7.B).

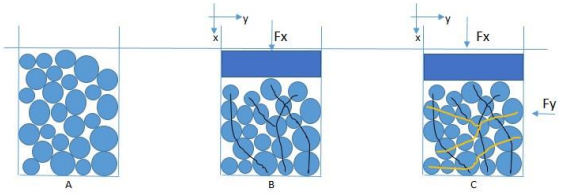

Figure 6.7 – Transmission of stress among the particles at different steps of deformation during compaction and densification.

Normally, in the feed mix, there are particles with various geometry. If particles are not spherical, for example, flake-like, they will orient in their preferred and random orientation during compression. However, during the compaction of the feed mixture system in the die, the sidewalls of the die can influence the compression of the system in the *y* direction (Fig. 6.7.C). In this way, the new particle orientation is possible to happen horizontally (Fig. 6.7.C). Thus, new contacts between particles in the compression direction are created. This happens if particles in the feed mixture allow the creation of more stress-chains with particles that are horizontally oriented from the vertical chains. Possibly, the moving of such systems could allow flow developed by the slip-stick behavior (Fig. 6.8). The slip-stick friction occurs when particle surfaces are sliding against each other or against the wall of the die. Such behavior is common among spherical particles.

During the slip-stick friction and all through a "stick" period, the shear stress rises until the static friction has reached a maximum pressure at incipient flow (p_{max}). Then, the densified and compressed system starts moving together along the direction of the die where suddenly decreased shear stress (τ) is replacing the static friction with kinematic friction. Hence the displacement in the x direction would show a peak of mass (m) velocity. The reason for sudden acceleration is that instantly, at the fraction of the mass movement, a portion of the force (F) is needed to overcome the reduced friction. That causes the additional energy, which is previously stored as elastic energy in the spring, to create the force which will accelerate the mass. This is very common to observe by using single-die pelleting devices with a blank die and particles that are difficult to interlock or bind with each other (Fig. 6.8).

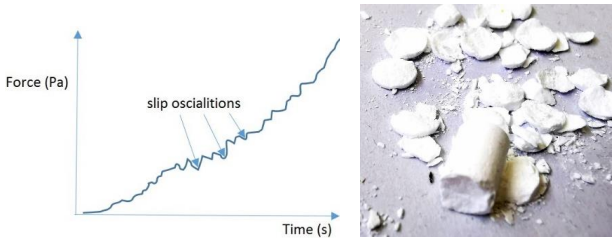

Figure 6.8 – Slip-stick oscillations and their consequence on the particulate material with low binding abilities

It is vital to understand these slip-stick properties of various ingredients that are part of the feed mixture powder system for probable prediction of the deformation of the feed mixture during pelleting. This also may be a tool for predicting the physical quality of the animal feed pellets. Dominant deformation that is usually happening in the x direction may define the final structure of the compacted material. In the continuous pelleting process the flow at constant volume and constant stress is called the steady-state flow, which is preferable in commercial feed manufacturing. Yet, before the steady state, shearing between the particles and between the particles and the wall of the pelleting die is necessary to occur. That is why the stress chains which are oriented during dominant deformation would define the structure of the final feed pellet. Stresses applied in formerly explained directions in a feed mixture powder system are helping to achieve the anisotropic properties of the material. Usually, the properties of the compacted material can defer, even if the same powder material would be used if moved in different and not predicted directions. This is usually neglected by process designers and feed formulators when different raw material is used to produce a cheaper feed. The steady-state flow, as the process of pelleting, leads to more anisotropic compacts than uniaxial compression with isotropic deformation that happens during tableting. Therefore, there are different physical properties of the finite compacted product between pellets and tablets even by applying the same forces.

6.11 Shear deformation and shear zones

The ideal compression in x direction and immediate dilation in y direction is called "pure stress". Such ideal deformation of the feed powder system does not normally happen during the pelleting or tableting process. The shear stress (τ) is low in the feed powder system during

initial compaction when the compaction volume is large. However, the consolidation of the particles increases as the shear stress increases and becomes high enough for the steady-state flow to be achieved.

The initial compression of the powder feed mix gives the particles high mobility due to the weight of the particles. Loose particles present in the feed mix tend to expand during densification and prior to initial compaction. This is happening because the particles of the system move over the other particles in the compaction systems. As explained by Török *et al.* (2003) in Schulze (2008), prior to steady-flow development, a decrease of the shear stress with increasing deformation within the feed mix leads to the concentration of the shear zones. During shear deformation, under constant stress, the steady-state flow overcomes the shear zone over the entire powder system of the feed mix. With increased shear deformation, the shear zones are concentrated in a region of decreased density. During shear deformation by the shear stress while compacting the feed powder mix, the shear stress first increases to its maximum, and thereafter decreases. However, if the constant shear stress dominates during pelleting the displacement in the x plane of the confined pelleting die volume, the maximum shear stress could decrease. Such a decrease would happen only if the filling rate of powders during pelleting is not constant.

The height of the shear zone has a large influence on the shear zone (*hs*) and on the shear stress (τ) as particle-to-particle interaction or particle-to-die-wall interaction. Such an effect creates dependence of the shear stress on the shear rate. Also, it creates dependence of the shear stress on the shear deformation at a certain shear height. Such deformation is happening if the shear height of the shearing zone has a relative velocity of the compression in the x plain exactly between the top and the bottom of the compressed feed powder. In random homogeneous feed mix powder systems, it is difficult to comprehend which of the ingredients present in the feed mix would influence most the creation of the shear-stress zones. The most realistic particles found in the feed mixtures are typically having many different shapes and sizes. To understand the phenomenon of particle interactions in complex animal and fish feed it is vital to experiment with the two-phase systems. These phases can have different physical or chemical properties, such as particle size, shape, density, or composition. The two phases may be present in different proportions and can interact differently within the system, leading to unique characteristics and behaviors. Such systems need to be developed preferably by the constituents which are majorly present in the homogeneous feed mix. This would provide valuable information on the creation of the shear-stress zones and hence the creation of the shear-stress in the compacting die. To compress two-phase powder systems, the large clusters and voids of the homogeneous feed mix are stabilized by weaker van der Waals forces. Those forces need to collapse so that a denser structure will be formed. Any further densification is created by rearranging particles within the contact areas by higher stress and minor changes in particle size. As explained by Andersson *et al.* (2008) the heterogeneous two-phase powder system interaction is dependent on particle size and particle size distribution, humidity, presence of liquids such as oil, or others. Displacement and diffusion of two-phase systems could have a diffusive or caging regime (Fig. 6.9). By analyzing the displacement and long-term memory of the materials, insights into the correlation structure and predictability of the pelleting process can be

achieved. The Hurst exponent can be used as a measure of long-term memory of the pelleted materials.

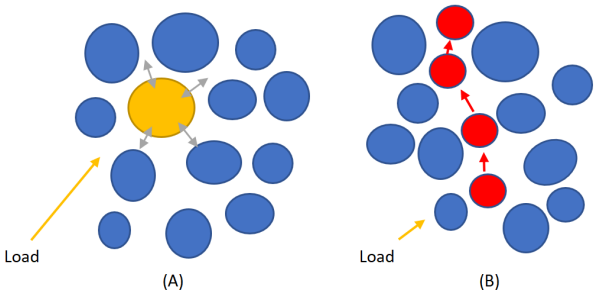

Figure 6.9 – Diffusive (A) and Caging (B) regime during the two-phase heterogeneous interactions.

The use of the Hurst exponent in feed pelleting helps in understanding the degree of self-similarity or fractal behavior of the feed particles exhibited by the time related to the pelleting process in the die. In feed pelleting, the Hurst exponent can assist in evaluating the efficiency and quality of the pelleting process. It can help identify patterns, trends, or dependencies which can be indicative of optimal conditions for achieving desired pellet properties, such as density, durability, and uniformity. By analyzing the long-term memory of the process variables, adjustments can be made to the pelleting parameters to improve the overall pellet quality and production efficiency. As explained by Andersson *et al.* (2008), by decreasing the Hurst exponent, more disordered density distributions will be formed and thus such structure can pack better. However, by decreasing the Hurst exponent it causes a rougher and more disordered interface between two-phase heterogeneous powder systems. If such an interface is formed, more sliding contacts and lower friction can be observed, and thus low compressibility between the two-phase heterogeneous powder systems (Fig. 6.10). All this contributes to nonlinear stress-strain behavior.

A B C

Figure 6.10 Ordered and disordered density distribution structure based on decreased (A), medium decreased (B), or increased (C) Hurst exponent.

Density distribution usually occurs across the shear surfaces, between the die wall and the closest layer of the feed mixture powder system. Generally, it is well-established knowledge that the force networks and chains of connected particles play a very important role in the

physics of powder materials and the final physical quality of compressed granular-based materials (Hinrichsen and Wolf, 2004).

6.12 Compressibility, compactibility and recovery of powder systems in the pelleting die

Die compaction is a common technology across many different industries. Commonly, animal feed products are pelleted. The die compaction, as a continuous process, normally involves filling of formulated feed mixture in the die, compression between the rollers and the ring or the flat die, and finally, ejection of compacted pellets out from the die. The scientific investigation of compaction based on compressibility and compactibility of different types of formulated feed is necessary to understand and overcome problems in the pelleting process. Also, the scientific investigation shall indicate optimal processes to produce physically good quality feed pellets. Compressibility refers to the ability of the powder to change in volume when subjected to pressure (Ilic *et al*. 2009), whereas compactibility is the ability of powders to convert from small particles into coherent solid dosage forms (Yap *et al.* 2008).

When powder systems are subjected to the loads, depending on the applied load, the rate of densification changes. The density of final compacts is greatly dependent on pressing methods, for example, die pressing or hydrostatic pressing may define the state of the applied stress. During the compaction of the powder feed material, the pseudo-plastic properties of the material prevail. The powder densification is most represented by the die compaction (Heckel, 1961). Some disadvantages during the die compaction process could significantly influence the density variations due to die-wall friction. As already mentioned in the previous text of this manuscript, the development of the mean density during die compaction can be assumed by measuring the compact height and the force applied to the feed powder. During the powder compaction cycle, the rise of friction is present between powder particles and the compacting-die surfaces. Also, friction during the unloading and ejection stages is of vital importance for understanding the final density of the compacted material (Brewin *et al.* 2008). During the die-unloading stage, the compression loads are stress-free, and elastic recovery of the compressed material is taking place. However, this recovery is resisted due to presence of the friction between the compacting-die wall surfaces. Friction is present due to the existence of the remaining stress within the compacted feed material, which is normally oriented toward the die-wall surfaces. As explained by Brewin *et al.* (2008), at the end of the die-unloading stage there will remain a plane over the pellets which is beyond the friction force between the powder system and the die-wall surfaces. This friction force will equal the recovery force within the compacted pellet. In the case of cylindrical pellets, one layer of the pellet will string back. That layer is leaning towards the side where the applied stress is present. This happens because the recovery force (A) of the layer within the powder-compact exceeds the friction force (S) that is present over the zone above this plane (fig. 6.11). Therefore, the die-unloading friction can lead to further densification of the powder system being compressed (Gethin *et al.* 2008).

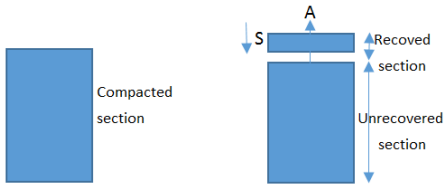

Figure 6.11 – Compact recovery on relaxation of compressed loads. Reproduction from Brewin *et al.* (2008).

Before the compaction will start it is assumed that the die-fill or density of the feed mixture in the die is consistent. However, this is very unlikely to happen due to factors beyond the topic of this Ph.D. thesis work. Burch *et al.* (2008) measured the density distribution of the initial powder bed in filled compaction dies and found a variation between densities of 10% to 15%. This was also measured by Gethin (2004) and the authors concluded that two regions with densities differing by 10% exist prior to compaction and after the die-fill. This study showed that for simple shapes, such as the shape of the deep cylinder, there is a negligible variation in the final density distributions. Such variation happens even for different vertical die-fill density distributions with the same total mass of powder material that is filled in the die. However, such a difference did not influence the final loads at the end of the compaction.

During the feed pelleting process, a feed mixture powder system is loaded mainly by compression where the compressive stresses and strains are positive. When assuming uniaxial compression on the compacted feed mixture a multiaxial stress applied on that mixture would be expected. According to Cocks (2008) under multiaxial conditions, during the compaction of the feed mixture, the three normal components of stress and three shearing components must be considered. However, during the compaction of the three-dimensional powder systems in the cylindrical die, only two stress variations are occurring. Those stresses are the hydrostatic pressure (p) and the von Mises equivalent stress (q). Considering that a feed mixture in the cylindrical die is subjected to axial (σ_a) and radial (σ_r) elements of the stress the multiaxial state of stress is unavoidable. The hydrostatic stress is the mean value of the three major stresses (one axial and two radial) where the asymmetric stress state is calculated as:

$$P = 1/3 * (\sigma_a * \sigma_r) \quad \text{Eq. 6.16}$$

Applied axial and radial components of the stress would also influence the strain (ε) on the total three-dimensional asymmetric cylindrical powder components of the feed mixture system (Fig. 6.12).

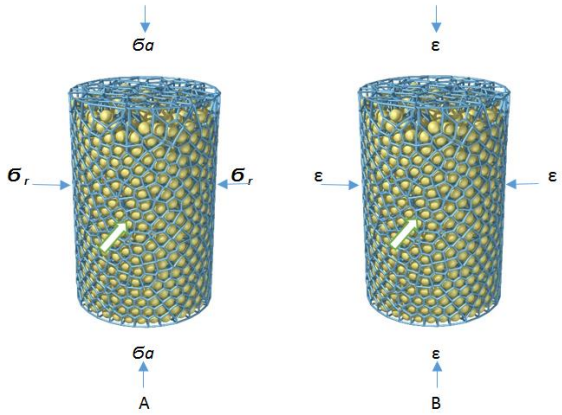

Figure 6.12 – Axial and radial components of stress (A) and strain (B) on axisymmetric powder system during compaction. Based on Cocks (2008).

The loading (Fig. 6.12 A) has the Mises equivalent stress related to major shear stresses defined as:

$$q = (\sigma_a - \sigma_r) \quad \text{Eq. 6.17}$$

The axisymmetric condition of Figure 6.12 B represents the axial and radial strains under axial and radial loads. These strains can be defined as the volumetric strain (ε_v) and the equivalent strain (ε_e), explained in eq. 6.18 and 6.19, respectively.

$$\varepsilon_v = \varepsilon_a + 2\varepsilon_r \quad \text{Eq. 6.18}$$

$$\varepsilon_e = 2/3\ (\varepsilon_a - \varepsilon_r) \quad \text{Eq. 6.19}$$

Compacted feed mix shows the elastic properties where particles are uniformly distributed in all orientations and can identify the response, defined as the relationship between stress and strain, by volume change (ε_v) presented in eq. 6.20 The effective stress leads forward to a shape change of the feed mixture powder system, shown in eq. 6.21.

$$\varepsilon_v = p/K \quad \text{Eq. 6.20}$$

$$\varepsilon_e = \sigma_e / 3G \quad \text{Eq. 6.21}$$

Where: p – pressure; K – bulk modulus; G – the shear modulus

If the response of the feed mixture powder materials would be uniaxially loaded the yield stress below the elastic response can be taken into account. However, this would be incorrect due to multiaxial loading influenced by the three-dimensional structure of the feed pellet. Therefore, it can be concluded that the pressure (p) in three-dimensional feed mixture powder systems

affects changes in the volume of the feed pellet. The stress, however, influences the shape change which is characterized by the effective accumulated strain in a three-dimensional feed pellet.

6.13 Physical and mechanical pellet quality

It is vital to understand the behavior of the feed particles compacted in the pellets due to the assessment of the final product's quality and possible prediction of failure in manufacturing. The physical and chemical behavior of feed pellets depends greatly on the amount of water that is present during the pre-compaction stage in the feed mixtures. Water is not dictating only the physical properties of the feed pellets but also the biochemical degradation, and microbial activity in the products (Lewicki, 2004). The way how the water molecules are bound to the internal structure of the feed mix powders and the degree to which water is freely available can be valuable information to understand the water activity (a_w) of a feed mixture during pelleting or in the downstream processes after pelleting. The a_w of powders can be defined as the equilibrium relative humidity of the material. When a feed mix powder comes into equilibrium with its environment prior to compaction the a_w in the powder is like the relative humidity of the atmosphere found in the feed mixture. Therefore, the feed mixture powder does not gain nor lose moisture over time. The steady a_w values are very important for assessing the phase transition on various temperatures of the feed mixture during and after pelleting. This is equally important for assessing the shelf life and physical quality of the pelleted product.

6.13.1 Physical properties of feed pellets after pelleting

Undesirable breakdown of compacted feed mixture can happen by fragmentation or abrasion during the transport of the feed pellets in the feed mill prior to packing or at the animal farms. This can further lead to dust formation and thus loss of the product in the system. The assessment and the physical property characterization of the feed pellets are very important and not an easy task. Porous feed mixture particles during the pelleting process are subjected to compression of multiaxial stress history. A better understanding of the structural changes that occur during pelleting of the feed particles at the molecular level would enable an effective understanding of the physical properties of pelleted feed products. This can also bring better assessment of the production parameters and hence optimize the physical properties of the final feed product. Multiple scientific techniques are available to provide valuable insights into the structural aspects of feed pellets. These findings can then influence further decisions or modifications to commercial technological approaches, thereby enhancing the physical quality of the product to better suit its intended application.

Among many techniques, a tensile strength analysis is a compression test that is performed by a Brazilian test (Adams and McKeown, 1996). Such test can detect the failures that occur very often during feed packing, transportation, or unloading at the farm. Also, failures can arise during feed pelleting where the changed density of the feed mixture powder can influence the cohesion of particles and consequently physical damage to feed pellets. To assess the failures before and during compaction as well as during ejecting the feed pellets from the pelleting die, some physical quality analyses are necessary. Except for the Brazilian test, other physical quality tests for compacted feed pellets are texture analyses and durability.

Brazilian test may evaluate the texture properties of the feed pellets through hardness analyses. Hardness analyses are comprised of tensile stress test, diametral compression, and simple compression. For feed pellets, one of the most convenient analytical approaches is the diametral-compression test, which includes the uniform tensile stress (σ_d) development across the loaded diameter (d) (Kergadallan *et al.*, 1997; Gethin *et al.*, 2004). Equation 6.22 explains the diametral compression test:

$$\sigma_d = 2_{F/}\pi D_t \quad \text{Eq. 6.22}$$

Where:

F - applied load

Dt – sample diameter or thickness

The diametral compression test consists of one dynamic cylinder that mechanically moves toward the feed pellet specimen. The feed pellet that is placed on the fixed disc is being compressed. The type of failure and the failure stress depends on the shape of the surface used for loading the tested material (Sinka *et al.* 2004). Failure stress expressed in MPa and the relative densities of the compacted pellets are greatly influenced by the length and diameter of the pellet (Brewin *et al.,* 2008). The simple compression test is also used to determine the physical properties of the material by applying two opposite forces and one coaxial compressive force (Fig. 6.13).

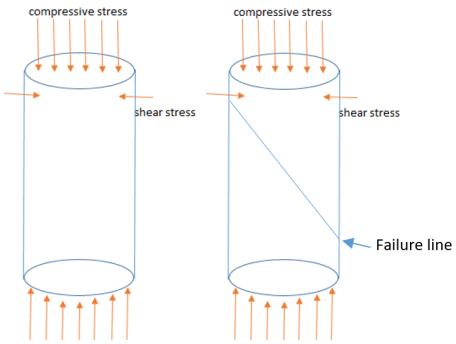

Figure 6.13 - Compressive stress and shear stress without the failure line and with the failure line.

During the compaction process, friction is exhibited between all surfaces at all points of the mechanical contact within the feed pellet. It is important to assess the influence of friction for predicting the final product quality.

6.13.2 Wetting and surface phenomenon of compacts

The surface phenomena are very important during the processing of feed powders and during intended usage. The physical properties of the pellets and stability of the pellets are mostly decided during compaction. A large fraction of compacted feed products consists of different materials with various phase boundaries and interfaces. Understanding the interfaces of different materials could evaluate the absorption of different liquid substances added to the feed pellets after pelleting. This could predict the colloidal interaction forces between structural elements in the compacted pellets and the liquid substances. The surface tension and the attractional forces are present between all molecules. When a surface of a pellet is created by compaction it takes a long time for molecules at the surface to orient differently from previous orientation. The liquid adsorbent adsorbed on an air-solid interface could happen as the adsorption of water from moist air or oil in the downstream oil coating processing. The adsorption at the solid surface of the feed pellet is happening at predetermined adsorption sites that can bind the adsorbate molecules.

Wetting and surface phenomena of the feed pellets are important to understand the contact angles (θ) of the adsorbents as for example oil or water and the pellets. When compaction of the feed mixture powder influences different densities in the feed pellets this can lead further to the formation of different surface properties of the pellets. This is usually happening when adsorbents are added to the feed pellets in the post-pelleting phase defined as top coating or vacuum coating. The surface contact angle between the adsorbent and adsorbing medium is based on three-phase boundaries: air, liquid, and solid. The equilibrium of those three phases is necessary to achieve the point lines of the contacts between the pellet surface and added liquids that can be described as wetting. If $\theta = 0°$ it can be concluded that the pellet is fully wetted and thus the liquid will spread in a thin layer over the entire surface of the feed pellet or it can be fully soaked in the pellet. Another extreme could be that $\theta = 180°$. In this case, it can be concluded that the feed pellet cannot be defined as wetted. In such a case, the oil or water droplet is theoretically "hovering" over the feed pellet, and it is almost with no contact with the pellet surface. The interfacial tension between the air and the liquid is balanced by the elastic reaction forces at the surface of the feed pellet. Non-Newtonian liquids, e.g., starch suspensions, are the only ones to engage these forces. The Newtonian liquids, e.g., oil or water will spread over the feed pellet with gravitational help, ideally until it gets to one molecule thickness. During the capillary displacement of a liquid in a porous feed pellet filled with air, the air will be displaced by the liquid if the contact angle measured in the liquid is equal to zero value. Adsorption and desorption occur at the surface of the interface between the feed pellet and the fluid-atmosphere which is surrounding it. A good example is pelleted shrimp feed which stays for a long time submerged in the water prior to the shrimps would start eating it. Such conditions of mass transfer are recognized as solid-liquid boundary surfaces. When molecules of water are attracted to the surface of the submerged shrimp feed pellet, they will start to adhere to it. Adherence in this case is called the adsorption of the fluid to the solid. In the case of desorption, the fluid molecules are escaping from the solid matrix, the feed pellet. Such desorption could be present, for instance, during the drying process of the feed pellets. Under the drying process, the free water molecules at the surface of compacts start to escape into the relatively dry fluid atmosphere, dry air. Such escape of molecules will happen until

reaching the equilibrium of relative humidity. Adsorption or desorption of the fluid is ruled by the physical and chemical characteristics of the feed pellet and its surface (Fig. 6.14). Adsorption and desorption are dynamic states where molecules are leaving the surface in a desorption process, while other molecules are getting attached to the surface in an adsorption process. Equilibrium is reached when the number of molecules departing from the surface and those being attached is similar and remains averaged.

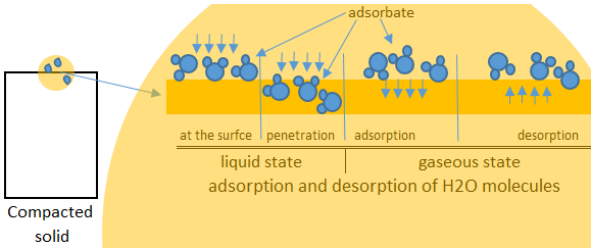

Figure 6.14 - Adsorption and desorption equilibrium of the water at the surface of the feed pellet.

Molecular adsorption in pelleted feed is the primary cause of swelling of the starches and changing the orientation of non-starch polysaccharides in the feed mixture or the feed pellets. The molecular adsorption process comes through the mechanisms of bound water, whereas capillary adsorption corresponds to free water. When the pellet is having low water activity, water molecules adhere easily to the surface of the feed pellet and its molecular structure. The water molecules are drawn into the network of the pellet wall only when the distance between the water molecule and the pellet becomes small enough. When the moisture of the feed pellets increases, the molecular attraction reduces, and therefore the volume increases. The volume increase of the pellet is closely equal to the volume of added water. Therefore, the deterioration of the compacted feed pellets can occur. In the case of shrimp feed pellets, it is undesirable to bring water activity to very low levels due to fast irreversible damage from adsorption compression when pellets are stored prior to feeding or submerged in water during feeding of the animals.

The surfaces of the feed pellets are having porous structures with small cavities that promote water transport in, or out, of the feed pellet by capillary adsorption. This is happening when cavities in the structure of the pellets are optimally sized to hold water by forces of the surface tension. The addition of nano components, such as enzymes, or micro components as lignosulphonates, during the feed mixing, can change the ability of feed pellets to absorb water and hence change the product quality (Fig. 6.15).

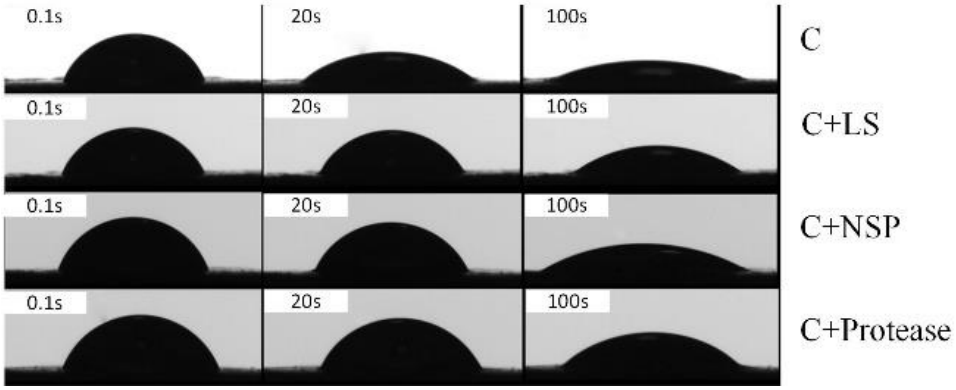

Figure 6.15 Sessile water drop and contact angle (θ) measurement during different time intervals at the surface of compacted microalgae (C) with added lignosulphonates (LS), xylanase (NSP) and protease. Source: Salas-Bringas *et al.* (2015).

The measurement of θ presented in Fig. 6.15 is called drop-shape analysis (DSA). The DSA analyses could be performed by using an optical instrument, with the image processing software, where the shape of a single drop can be analyzed and evaluated.

6.13.3 Water activity and hygroscopicity

Feed pellets produced with various combinations of feed materials will behave differently when the humidity is changed from lower to higher values. The adsorbed moisture content versus water activity (a_w) could be measured by the eq. 6.23.

$(1/x_w) \cdot (a_w/1-a_w)$ Eq. 6.23

where:
x_w - moisture
a_w - water activity

Except for changes in the physical properties the a_w values can influence the population growth of microorganisms. Lower a_w limits for microbial growth are defined as such:

Bacteria (a_w) 0.91 – 0.95
Yeasts (a_w) 0.88
Molds (a_w) 0.80
Halo-tolerant bacteria (a_w) 0.75
Osmo-tolerant yeast (a_w) 0.70
Xero-tolerant molds (a_w) 0.65

The relative rate of different spoilage reactions as a function of a_w is presented in Fig. 6.16

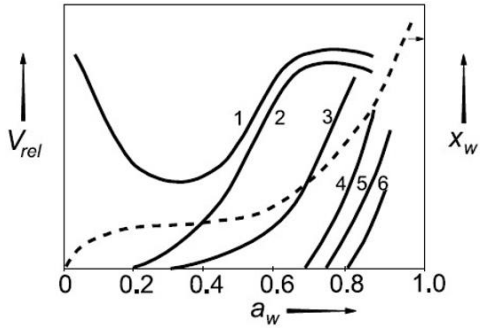

Figure 6.16 - Relative rate of different spoilage reactions as a function of water activity a_w in food. **1:** lipid oxidation, **2:** browning reactions, **3:** enzymatic reactions, **4:** molds, **5:** yeasts, **6:** bacteria. The dashed line indicates the sorption isotherm of the sample material. Adopted from Figura and Teixeira (2007).

The sorption isotherm and its shape could predict the quality of physical characteristics as a function of a_w. The sorption isotherm is divided into three regions of water bonding states (Fig. 6.17), where region I have a very low a_w and moisture content.

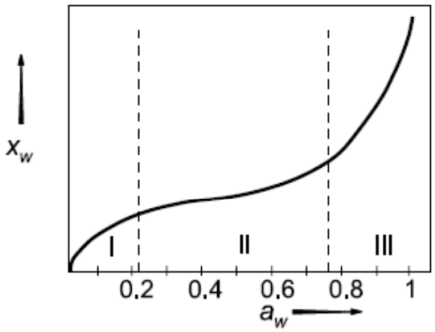

Figure 6.17 - Sorption isotherm graph showing three regions of water activity. Source: Figura and Teixeira (2007).

In region I water is completely bound and unavailable to contribute to any kind of change of the pelleted feed material. Most feed pellets in this region are hard and brittle. It is a very rare occurrence that the dry feed pellets will be in the region I. Most of the finished feed pellets are out of region I and within region II. In region II the a_w is low enough, thus the spoilage reactions cannot proceed if the enzymes are not existing. This region is good for dehydrated pellets intended for long-term storage. Region III is the region where uptake of the moisture happens rapidly. The moisture content comes to the levels where the water can be free and thus support chemical and biological reactions. In this region, the feed pellets show elastic and semi-plastic behavior.

6.14 Significance of rheology research in feed pelleting

The deformation and flow of feed material under applied stress are adequately studied in the science of rheology. Rheology is used to study the flow and deformation of feed raw materials and compacted feed pellets under different conditions, such as temperature, pressure, and shear. In feed pelleting, rheology research helps to understand the behavior of the feed materials during pelleting, extrusion, or transportation. Any change of behavior may affect the nutritional and physical quality of the final feed product. Studying how materials flow and change shape, called rheology research, is important for making animal feed. The science of rheology can help feed manufacturers to optimize feed manufacturing in order to gain better mechanical pellet quality. Such research can help optimize the feed pelleting process by providing insights into the behavior of the feed materials under different conditions. This can lead to the development of more efficient pelleting methods, resulting in higher technical and nutritional quality and more consistent feed products. All this can lead to manufacturing quality feed products with lower prices and an overall better economy. Rheology research can help in understanding the factors that influence pellet durability and hardness as indicators of optimized mechanical feed quality. By studying the rheological properties of the feed materials, researchers can identify the ideal conditions for producing optimal durable and stable pellets. Hence, rheology can facilitate control of the quality of the feed pellets by understanding the factors that affect their physical and chemical properties. By knowing the rheological properties of the feed materials, researchers can develop strategies to optimize and control the factors such as pellet density, size, and shape, which can affect the nutritional value of the final product. Optimized feed manufacturing can improve overall feed quality, reduce feed wastage, and enhance animal performance. However, the feed manufacturing process can be challenging due to the complexity and variability of feed materials found in the formulated feed, which can alter pellet quality and production efficiency. Therefore, it is needed to study the flow and deformation of materials under stress during and after pelleting. Understanding the physical properties of feed materials in the ever-changing composition of the animal feed, such as their viscosity, elasticity, and plasticity can optimize their behavior during pelleting too. By measuring and analyzing these properties, investigators can optimize the formulation and processing parameters of certain novel feed materials. In such a way, the investigators may identify and address problems that may arise during the pelleting process, such as clogging of the pelleting die, bridging of the material or pelleting die wear. By understanding the underlying causes of these issues, the feed industry can develop strategies to improve pellet quality, reduce downtime, and increase profitability.

7 Hypotheses and aims of the Ph.D. thesis

To understand the main existing issues in feed manufacturing it is vital to investigate and isolate the important factors that may influence further development. It is hypothesized that the understanding of the rheology of novel feed ingredients is rather poor. Thus, there is inadequate knowledge of the rheology of feed materials deriving from unicellular organisms. The importance of offering future directions of the technology, methods, and novel feed ingredients for better management in the feed mills has been prioritized in **Paper I**.

Experiment I, **Paper II** have been designed to examine the hypothesis that the pin-pointed novel feed ingredients from **Paper I**, microalgae, together with lignosulphonate, can affect the physical strength and decrease hydration of the compacted microalgal pellets. It was also hypothesized that microalgal pellets can affect swelling under stagnant water. However, the inclusion of feed additives based on lignosulphonate and enzymes can minimize pellet swelling. Therefore, the aim of **Paper II** was to investigate steps forward and to rheologically characterize the pellets made of microalgae and understand better their shelf-life potential during the storage and usage phase underwater.

In **Experiment II**, **Paper III** the objective was to study the hypothesis that different enzymes and their dosage can lower the pressure at anticipant flow when a novel material based on microalgal biomass is pelleted by a single die pellet press. Furthermore, the hypothesis that both novel materials and different dosages of enzymes may reduce the tensile strength of the pellets was examined. Also, the hypothesis was that the inclusion of enzymes in microalgal pellets can reduce the underwater pellet swelling and decrease the interaction of the pellet surface with oil and water. **Paper III** investigated these hypotheses.

Experiments III and **IV** in **Paper IV** were systematized to investigate the hypothesis that a novel feed ingredient, the yeast *Cyberlindnera jadinii*, as a replacer of the fishmeal in the whiteleg shrimp (*Litopenaeus vannamei*) diet with or without adding enzymes, can influence pressure at anticipant pellet flow out of the pelleting die by worsening pellatability and physical properties of the pellets. **Paper IV** investigated these hypotheses.

8 Materials and methods

To attain the goals of the Ph.D. thesis work, the investigational effort has been guided by several steps, as presented in the outline of this thesis (Figure 5.1). Those steps include:
- Screening and investigation of the current issues in the feed manufacturing technology,
- Identifying the novel feed raw materials where poor or none of the published work has been done regarding the technical quality of the feed pellets and inclusion of enzymes,
- Mixing of the novel materials with enzymes,
- Single die pelleting, and
- Analytical methodology development and/or introduction for the assessment of technical pellet quality.

8.1 Screening of the current issues in feed manufacturing and identification of the novel raw materials worth exploring

Among many different issues occurring in an ever-changing approach to feed manufacturing, the need for novel protein-based raw materials has been recognized as worth exploring. The focus was set on the physical quality of the pellets that incorporate novel protein-based raw materials. Microalgae and yeast were identified as novel materials of importance in terms of the physical quality of compacted feed pellets for livestock and aquaculture. Both microalgae and yeast are sustainable feed materials with low environmental impact, as they do not require large amounts of land, water, or energy to produce. As such, they have been explored as potential alternatives to traditional feed materials such as fishmeal. Also, the enzymes phytase, protease, xylanase, and endo-exo 1.3-beta-glucanase were used to explore their influence on the potential utilization of the novel raw materials. Enzymes can unintentionally affect the contents inside and outside of the cells that are being disrupted. Therefore, thorough optimization and evaluation of enzyme treatment were conducted. The novel materials and enzymes were chosen to explore their influence on the die-wall friction during pelleting, the final physical quality of pellets, and overall manufacturing aspects such as the pressure at anticipant flow.

8.2 Mixing process

The target of the mixing process during the Ph.D. study was to ensure the homogeneity of water, enzymes, and novel materials in the powder feed mixture to be pelleted. The hypothesis was based on the fact that some enzymes or combinations of enzymes in different dosages may decrease the friction between the powder feed mixture and the pelleting die wall. Such decreased friction may improve downstream process efficiency. However, decreased friction can also cause weaker packing of the feed particles and thus create a low physical quality of the pelleted feed product. Mixing of novel feed raw materials with the enzymes xylanase (0.01%) and protease (0.006%) in **Paper II**, and phytase, protease, and xylanase at 3 inclusion levels (**Paper III**), as well as the addition of an enzymatic cocktail, made of protease and endo-exo 1,3-β-glucanase (**Paper IV**), was done with the same mixing time of about 800 seconds. The mixing time was chosen to ensure a homogeneous mixture when the speed of the mixing

device was 250 rpm. and the mixing knife speed was 500 rpm. During the mixing of the novel raw material or formulated diets, 2.5% of distilled water (**Paper III)** or 10% of distilled water, were added to the feed mixture (**Paper IV**) to secure enough moisture for further enzymatic and manufacturing processes. Water was also used as a carrier of the minor enzymatic dosages so that homogeneity of the enzymes could be achieved throughout the entire mixing batch. Prior to pelleting, all homogeneous mixtures from each experiment were placed in vacuum-sealed bags and stored in the freezer at an average temperature of -20°C.

8.3 Steam conditioning of experimental mixtures

Paper IV describes the simulation of commercial steam conditioning. Each dosage of the mixed feed was weighted to be 0.13 g each and had a moisture level of 13%. Each feed dosage was thereafter placed in Eppendorf tubes which were sealed well and prepared to be conditioned by evaporating the free water found in the Eppendorf tube and between the feed particles. The steam forming in the Eppendorf tube happened when 30 sealed tubes were placed into the boiling water for 3 minutes. Thereafter, the tubes were placed at a temperature of 4℃ to cool down so that free water would condense rapidly within the entire feed mash present in the tube. Steam-conditioned feed mixture from a single Eppendorf tube was used to produce one pellet.

8.4 Pelleting of experimental mixtures

Pelleting process for the entire Ph.D. thesis was done with a single-die pellet press designed at the Norwegian University of Life Sciences, Ås, Norway. Single-die pelleting equipment was used to facilitate precise control of applied pressures, pelleting time, and temperature. Also, a very small amount of the material, about 0.2 grams, was used when pelleting with a single-die pellet press. The entire investigation was done with 250 grams batches for each experimental treatment. A thorough description of the pelleting process and the pelleting equipment is given by Salas-Bringas *et al.* (2010, 2011). Pellets were produced at the temperature of 81°C due to protection of the animal feed against salmonella and with respect to Norwegian regulation, VKM Report 2006: 20, 3B. To characterize the compressibility of microalgae pellets in **Paper II** the pressures of 7, 11.6, 23.3, and 35 MPa were used at a constant temperature of 81°C. Thereafter the chosen pressure of 12 MPa and the load of 285 newtons with the compressing speed of 10 mm/min were used during pelleting in **Papers III** and **IV.** The pellet diameter for all treatments was 5.5 mm. The total retention time of the materials in the compressing channel of the blank die was 9 minutes for all the experiments and all the treatments.

8.5 Physical pellet quality assessment and analytical methodology

A short overview of the methods used for assessing the physical pellet quality and surface properties is presented in Table 8.1.
Regarding the aspect of developed analytical methods of work used in **Paper II** and **III,** the underwater pellet swelling method (UPS) with image analyses, must be pinpointed as a novel

analytical approach. Such methodology is particularly important for the precise measurements of the lifetime of pelleted products intended to be used in aquaculture production (**Paper IV**). To monitor the swelling of a pellet under stagnant water a specific arrangement including 3D printed platform for the pellet and the tweezer for placing the pellet onto the platform was designed and incorporated into a pre-developed glass container. Such an arrangement was incorporated into the commercial imaging analysis system, an optical tensiometer (**Paper II**). An optical tensiometer was also used for assessing the hydrophobicity and hydrophilicity of diametral surfaces of pelleted microalgal biomass (**Paper II** and **III**) and pelleted feed.

Table 8.1 An overview of the methods used for assessing the technical pellet quality

Quality parameter	Method description	The indicator for:
Tensile strength, hardness	Diametric compression test (**Paper II, III and IV**) by using force (F) in Newtons during a diametral compression at 1 mm/min	Stability of the pellets during handling and storage.
Pellet stability under water	Pellet swelling, image analysis (**Paper II and III**)	Pellet hydration and swelling under stagnant water.
Pellet surface interaction	Surface contact angle - image analysis and water or oil adsorption properties (**Paper II, III and IV**)	Water or oil-repellent properties. Changed interaction of the pellet with oil and with water due to enzymatic treatment of microalgal biomass and/or replacement of the fishmeal with yeast.
Water activity	Measuring the ratio of the vapor pressure of water in the substance to the vapor pressure of pure water at the same temperature and pressure.	Growth of microorganisms, enzyme activity, and chemical reactions that affect product quality and storage stability.
Particle size distribution	Malvern Mastersizer, determination of the particle size distribution in the feed mixed mash (**Paper III and IV**)	Improved availability of the mixed feed particles during steam conditioning and compaction during pelleting. A higher surface area leads to higher compaction and densification and lower dissolution underwater.
Pellet surface roughness	Surftest SJ-210 as a numerical scale of the surface condition influenced by novel materials or the enzymes with diverse packing-ability of the particle in the pellets (**Paper IV**)	Uneven or slow material flow in processing equipment. A smooth pellet surface can reduce friction and improve flowability during pelleting. Also, the impact of the dissolution rate of the pellets under stagnant water and physical pellet quality where smooth pellets may have better packing of the feed particles and thus withstand longer the attrition during transport.
Measuring of soluble protein and phosphorous	Spectrophotometric method (**Paper III**)	Changed chemical properties of the microalgal biomass due to enzymatic treatments.
Pressure at initial flow measurement (*p-max*)	Measuring maximum pressure required to initiate compressed pellet to flow through a pelleting die (**Paper II, III and IV**)	Friction between the die wall and the pellet surface that may lead to more energy consumption during pelleting.

9 Results and Discussion

9.1 Raw materials for future challenges in the feed production

Novel feed raw materials and their nutritional and rheological variability must be understood prior to animal feed being formulated and certainly before feed manufacturing. Achieving sustainable, efficient, and flexible feed manufacturing must include a good understanding of the rheological relationship between conventional and novel raw ingredients. Hence, the role of novel ingredients integrated into animal feed in the pelleting process is becoming very important. In **Paper I** it was concluded that there is often tension between the routinely used commercial feed ingredients and the high capital technological infrastructure that dictates the ways that animal feed is manufactured. The variation in the feed ingredients may be considerably high across different ingredients and within a single ingredient and its dosage. Without a complete rheological analysis of a single feed ingredient and its inclusion in the feed formulations, the production variability during feed manufacturing will continuously be the unknown factor that may endanger the process of sustainable manufacturing.
Inconsistent and unexpected processing may result in inaccuracies in technical, physical, and nutritional feed quality too. An optimal inclusion level of the novel feed ingredients can be used in the pelleting process when precise effects of processing are known. Adopting and developing analytical techniques are also rather important for measuring the physical properties of animal feed. The key elements from a feed manufacturer's perspective and the criteria for potential inclusion rates, stability, handling, and energy consumption are all important when measuring pellet quality. Various changes during the feed pelleting process may happen due to the chemical composition of the raw materials previously defined as by-products, and their thermomechanical properties in relation to the effect on pellet manufacturing (**Paper 1**). This agrees with Bastiaansen *et al.* (2023) where the focus has been on the thermomechanical properties of novel ingredients that can adversely affect pelleting process with increased energy costs and decreased final pellet quality. Alternative feed ingredients should not increase energy consumption during feed manufacturing unless the inclusion of such ingredients reduces feed costs (**Paper I**). The usage of microalgae and yeast as protein sources instead of fishmeal, together with feed enzymes, may lower electrical energy usage considerably (**Paper III** and **Paper IV**). Nevertheless, this can influence the other factors defining the technical quality of the feed pellets such as densification, tensile strength, the surface roughness of the feed pellets, interaction of the pelleted material with oil or water, etc..

9.2 Pressure and its influence on density change in the microalgal pellets

Most of the powders when being compacted would change their density according to given pressures in the pelleting die. However, this density change is not observed when compacting the microalgal biomass beyond the pressure of 7 MPa ($p<0.05$) (**Paper II**). The compressibility of microalgae can be swiftly changed from the bulk density of 600 kg/m³ when compressed to 1400 kg/m³ by applying the pressure of 7 MPa. When applying any further pressure from 7 MPa to 35 MPa no further densification has been observed (**Paper II**). However, below 7 MPa the pellets were not sufficiently strong to keep a cohesive shape during handling, thus causing

severe breakage of the pellets. The cause for this observation, once the compacting load has been removed, may be attributed to an elastic relaxation in the microalgal biomass. Such relaxation or recovery is resisted due to presence of the friction between the compacting-die wall surfaces. This friction force, as previously explained, has matched the recovery force within the compacted pellet. Similar behaviour has been also observed in pellets made of compacted wheat gluten when compaction was done at temperatures and moistures over the glass transition (Salas-Bringas *et al*., 2012).

9.3 Effect of enzymatic hydrolyses on chemical change of pelleted microalgal material

Different dosages of phytase did not influence the changes in total soluble protein measurement when compacting the microalgal material in the pellets (**Paper III**). Perhaps a limited amount of water and conditions for the hydrolysis as temperature, pH and reaction time were not optimized during the experiment performed in **Paper III**. However, when experimental treatments with the phytase were compared to the control sample the soluble protein content was observed to be significantly higher in samples treated with phytase ($p<0,01$). Polyphosphates are of pivotal importance in microalgae (Grobbelaar, 2004), and their covalent binding to proteins enables microalgae to acclimate to various stress conditions (Sanz-Luque *et al*., 2020). However, according to the results presented in **Paper III** the experimental treatments did not provide any evidence on what type of enzyme or their dosage can influence the availability of phosphorous in the pellets. This is also confirmed by Xin *et al*. (2010) where the authors explain this as due to accumulated inorganic polyphosphate in *Desmodesmus sp.* remaining in the microalgal biomass, even after breaking the cell wall. However, by introducing the enzyme phytase, the hydrolyses of the links between polyphosphate and protein occur and may reduce the viscosity due to an increased level of soluble protein. Phytase is a very potent enzyme that can catalyse the hydrolysis of a wide range of molecules containing phosphorous (Oh *et al*., 2004). Phytase thus enables detachment of the phosphate from a wide range of molecules. p_{max} as a resistance to the flow of the pelleted material shows that the total phosphorous content in the trials did not influence the p_{max} of the pellets when pushed through the single die (**Paper III**).

9.4 Discharge pressure at anticipant flow (p_{max}) as a tool for assuming the electrical energy consumption during pelleting

Different enzymes and their combination had a significant influence on p_{max}. Phytase and xylanase showed to significantly decrease ($p<0,05$) the resistance of the compacted microalgal pellets during discharge (**Paper III**). However, all other enzymes, their combination, and different dosages did not show to significantly change the discharge pressure of the microalgal pellets (**Paper III**). The observed reduction of p_{max} for the recommended dosage of xylanase and all dosages of phytase may be explained by changes in the chemical structure of linearly layered polymers in the cell wall, well known as xylans. Xylanase may hydrolyse the bonds between xylans in the cell walls, decreasing the resistance to motion and thus friction in the die. Every enzyme in the combined enzymatic cocktails possibly had some influence on changing the rheological properties of microalgal biomass (**Paper III**). However, that may be

possible with changing the temperature of the medium or dosage of the enzymatic mixture. The role of xylanase can be explained by the hydrolyses of the xylans present in the microalgal cell walls. The role of protease was to influence the solubility of the protein. The role of phytase during hydrolysis was to cut the phosphate ester bonds. **Paper III** explains that there was no indication that mixtures of xylanase, protease and phytase, in different dosages, influenced p_{max} when compared to the control treatment ($p>0.05$). The reason for this unchanged p_{max} can be that the proteolytic activity of protease may fully or partially deactivate other enzymes by affecting the active sites of the enzymes. At those active sites, the protease may donate or accept hydrogens and destabilize the electrical charge build-up along the reaction mechanisms (Shafee, 2014). Also, possible reason for the unchanged p_{max} may be related to very low water content during hydrolysis in the experiment (**Paper III**).

In **Paper IV,** experiment 1, the flow resistance during discharge of the feed pellets did not show any characteristic difference ($p>0.05$) when adding either 2.5% and more up to 20% of the yeast *Cyberlindnera jadinii* to the feed as compared with control feed without added yeast. However, pelleting pure yeast, as a negative control, significantly increased p_{max} by 17 folds when compared to all other diets ($p<0.001$). In experiment 2 (**Paper IV)** it was observed that enzymatic hydrolysis in the feed with 5% yeast did not show any change in p_{max} ($p>0.05$). However, when enzymes were included in the feed formulations with 20% added yeast a significant increase in p_{max} was identified ($p<0.001$) when compared with the control feed with added enzymes. When yeast was the only medium to be pelleted (experiment 1, **Paper IV**), p_{max} showed to be significantly higher ($p<0.001$). This may be explained by the changed properties of powder systems affecting their densification (relationship between particles) and compaction (relationship between particles and compaction die). The structure of the solid powder materials is mostly determined by van der Waals forces between the small particles (<100 m^{1}). Because of such forces, the end structures are loosely packed and that could lead to process-related issues such as increased energy use during pelleting or blocking of the pelleting die. This is explained in detail by Walstra (2003). When only yeast was pelleted at high temperatures and pressures the deposited micro-scale material of the single-cell organisms possibly diffused from one particle to another through solid bridges. Thus, the forces influencing the cohesion of powder particles rested on the contact area of the solid bridges and their diameter. This may have influenced the build-up of a large area of a pellet with a high-density structure according to Pietsch (1991). A such large area may require high forces to be pushed through the channel of the die. A similar significant difference was observed in **Paper IV,** experiment 2, when 20% yeast was added.

9.5 Change of water activity (a_w) due to enzymatic hydrolyses of the novel feed raw materials

Enzymes added to microalgal biomass, such as protease, xylanase, and additive lignosulphonate, increased the water activity of the pelleted material (**Paper II**). Similar conclusions from **Paper III** are explaining that all the enzymes that were added to microalgal biomass, independent of their dosage levels, decreased a_w when added separately. There was only one exception associated with the phytase added as a double dosage that did not change a_w. However, when enzymes were added in the mixed form together, no influence on a_w was

observed, except for the half-dosage mixture of protease and phytase. Water activity was correlated to p_{max}, where increasing the water activity values also increased the p_{max} values (**Paper III**) which do not correspond with the general literature. It's important to stress that the relationship between water activity and viscosity can be complex and depend on the specific properties of the powder material. In this particular case, if the water activity becomes higher, the powder might become more sticky or exhibit the opposite flow behavior than expected. This could be explained due to the formation of a pseudo-paste-like mixture, which could give the increased viscosity. However, this is not a common scenario in powders with low water content, like the powder mixture in **Paper III**. It is very unlikely that this correlation is directly related to the basic effect of increased water activity on viscosity in the powder during pelleting. Therefore, the reason for this must be seen somewhere else beyond the particle level. Moreover, higher a_w significantly enhanced the tensile strength of the pellets (**Paper III**). Water activity did not show to be correlated to either oil contact angle and water contact angle, or the increased UPS rates (**Paper III**). Also, a_w change was not observed when replacing fishmeal with yeast in the formulated feed whether the novel material was treated with the enzymes or not (**Paper IV**). Enzymes can indirectly affect water activity through their catalytic activities and interactions with substrates or other molecules. Enzymes have specific active sites where substrates bind and undergo chemical transformations and can modulate the water activity in their microenvironment through water binding, dehydration, enzyme-substrate interactions, and conformational changes (Gutteridge and Thornton, 2004). Enzymes often undergo conformational changes upon substrate binding or during catalysis. These structural changes can modify the local environment around the active site, including water activity. For example, the movement of specific amino acid residues can expose or bury water-accessible regions, affecting the local water environment. However, the question remains, if an enzyme or mixture of different enzymes may produce such results in an environment with such a low water content.

9.6 Physical pellet quality change due to enzymatic hydrolyses of the novel feed raw materials

9.6.1. Tensile strength

Paper II explains that microalgal pellets show to have a brittle nature during the tensile strength analyses. This is clearly demonstrated by a typical stress-strain curve, where the pellets exhibit a sudden decrease in normal force and a distinct breaking point when subjected to tension. Such observation validates the estimate of the tensile strength from the peak force in diametral compression. Compacting pressure increased the tensile strength of the microalgal pellets ($p<0.05$), even if compacting pressure did not influence the density change in the microalgal pellets. The increase in compacting pressure makes stronger pellets under tensile stresses (**Paper II**). The addition of 0.006% protease and 0.01% xylanase, when calculated on a dry basis for the batch of microalgae, promoted a significant reduction of the pellet tensile strength. The addition of lignosulphonate did not significantly affect the tensile strength of the microalgal pellets. In **Paper III** it is shown that the tensile strength of pellets cannot be influenced by adding different enzymes, independent of their dosage. The same was observed

when the enzymes were added combined or as a single enzyme. No relationship between pellet strength and p_{max} was observed (**Paper III**). When 20% of yeast was added to the feed in experiment 1 (**Paper IV)** and compared to the control diet, and diets with 2.5% and 5% yeast, there was a significant increase in the tensile strength of the pellets. Also, when adding enzymes to the feed with 20% yeast in experiment 2, the tensile strength increased significantly ($p<0.001$) (**Paper IV**). This was not observed for other yeast-containing feeds with added enzymes when compared to the control feed.

Increased tensile strength of the pellets by over 9 folds was observed when pelleting only yeast without other ingredients (**Paper IV**). **Paper IV** shows that the tensile strength can be moderately correlated to p_{max}. Almost half of the tensile strength, as a dependent variable, may be explained by p_{max} during the discharge of the feed pellet from the die (**Paper IV**). A strong correlation between tensile strength and p_{max} was observed in experiment 2 (**Paper IV**) (Fig. 9.1).

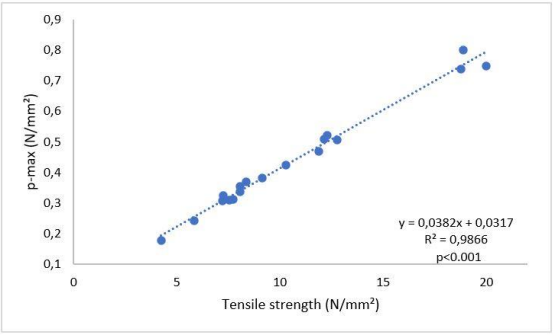

Figure 9.1 – Correlation between p_{max} and tensile strength

Pellet tensile strength showed to be increased 4 folds when adding up to 20% of the yeast in feed used in experiment 1 of **Paper IV**. In **Paper IV**, experiment 2, the enzymatic hydrolysis with a limited amount of water together with up to 20% yeast increased the hardness of the feed pellets. For 20% added yeast that the 2 folds increase was observed ($p<0.001$). The reason for this may be intense interactions and packing of 60% of the small particles under 100 μm in the feeds during pelleting in the single die. This also can be explained by very small particles in a certain ratio to larger particles that can substantially contribute to better physical properties of the pellets by interlocking bonds at micro-scales. Certainly, particle relations are dependent on the geometrical arrangements and porosity of mono-sized particles. The compactability of the experimental microalgal material might have been determined by particle size distribution, the shape of the particles, surface morphology, and the pore structure of the particles. However, this has been beyond the scope of this experimental work. Such dependency can influence the microalgal powder material to convert small particles into consistent solid structures (Yap *et al.* 2008). Densification is nothing else but the rearrangement of particles within the contact areas by higher stress and minor changes in particle size and shape. This can indicate that the heterogeneous two-phase powder interaction is dependent on particle size and particle size distribution, humidity, presence of liquids, etc. This may all influence the displacement and

diffusion of the powder systems which could have diffusive or caging dynamics of the smallest particles in the system (Andersson *et al.*, 2007).

9.6.2. Pellet surface roughness

Diametral and longitudinal surface roughness aimed to analyse the irregularities at the surface of the pellets. Diametral surface roughness was significantly different ($p<0.05$) when yeast was added to the feed as compared to the control feed with no yeast, except for the feed with 5% added yeast in experiment 1 (**Paper IV**). Longitudinal roughness analyses showed that by adding 20% of yeast to the feed there is lower roughness in comparison with the control pellets, pellets with 2.5% yeast, and pellets with 100% yeast. This indicates that by adding 20% yeast to the feed pellets it is possible to achieve the best compaction. That also can be concluded with a strong correlation between p_{max} and longitudinal surface roughness (Fig. 9.2). More than half of the longitudinal surface roughness, as a dependent variable, may be explained by flow resistance in the pelleting die during shrimp feed pellet discharge ($R^2 = 0.51$). However, the correlation between the diametral surface roughness and p_{max} was not observed ($R^2 = 0.07$). That indicates that adding yeast to the shrimp feed does not influence densification. Similar behaviour of different raw materials was observed in Salas-Bringas *et al.* (2012). **Paper IV**, experiment 2, shows that longitudinal surface roughness presented as the interaction between particles and the pelleting die wall is lowered in feed containing 5% and up to 20% yeast and added enzymes. The longitudinal surface roughness results from **Paper IV,** experiment 2, indicate a linear decrease of surface roughness along the longitudinal pellet wall by replacing fishmeal with yeast when the feed mash was treated with enzymes (Fig. 9.3). However, for diametral surface roughness, created by interactions between feed particles during compaction, the same can be concluded only for 5% and 10% added yeast in the enzymatically treated feed. No significant difference was observed in diametral surface roughness between enzyme-treated feed containing 2.5% or 20% yeast. Pellet surface roughness showed to be influenced by the particle size of the material, which is in line with Sarkar *et al.* (2014) and correlated with p_{max} during pelleting. In **Paper IV** the addition of enzymes was shown to enhance these effects.

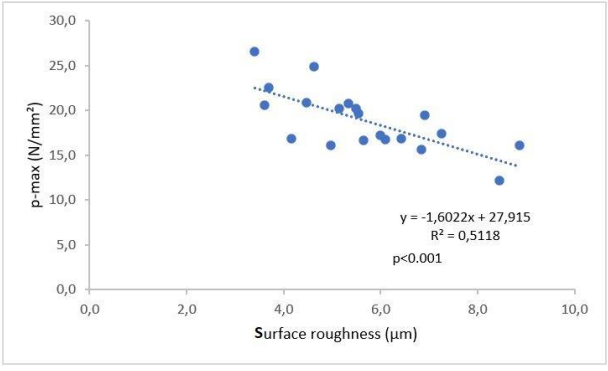

Figure 9.2 – Correlation between p-max and longitudinal surface roughness, exp.1, **Paper IV**

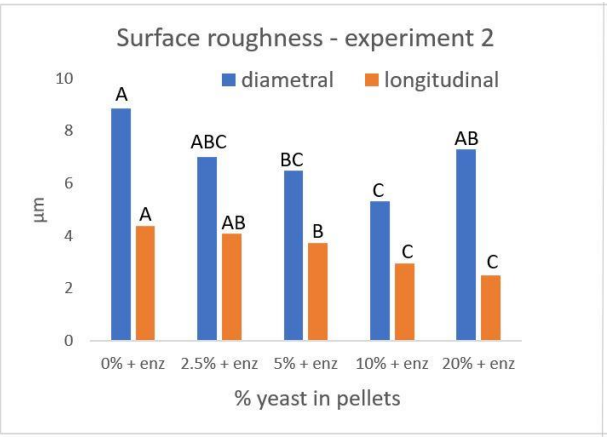

Figure 9.3 – Enzymatic hydrolysis decreasing the longitudinal and diametral surface roughness, **Paper IV**

9.7 Introduction of the non-conventional methodology for pellet swelling under water and pellet surface interaction with water and oil

9.7.1 Monitoring the swelling of feed pellets under stagnant water

The enzymes protease, xylanase (NSP), and additive lignosulphonate (LS) decreased the swelling of the microalgal pellets (**Paper II**). Protease produced the lowest swelling and the less hydrophilic pellets followed by LS and xylanase (Fig. 9.4). The values of the figure show how the microalgal pellets swell during the given time frame. Protease influenced the prolonged cohesivity of pellets, whereas enzymatically non-treated microalgae had the fastest swelling.

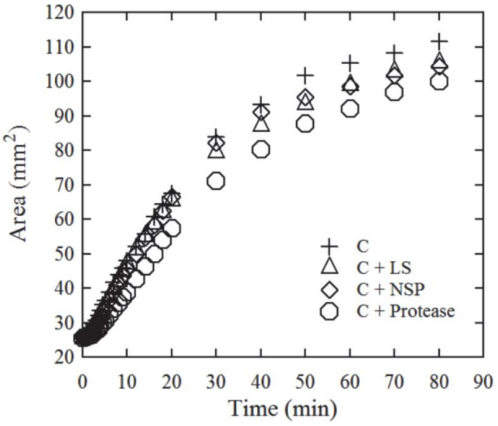

Figure 9.4 – Mean values of diametral measurement in the cross-sectional area of microalgal pellets by the time of the analyses, **Paper II**

Pellets treated with a single enzyme or mixture of enzymes decreased the swelling speed when compared to the control treatment (Fig. 5, **Paper III**). The pellet swelling was 30.7% slower for the first 60 seconds when the microalgal material was treated with phytase compared to the control treatment. When the combination of protease and phytase was used to treat the microalgal material and when pellet swelling was observed within the first 60 seconds, the swelling rate was lower only by 1.7% when compared to the control treatment. However, the lowest pellet swelling was observed at 14 minutes for pellets where the microalgal material was treated with the mixture of xylanase, protease and phytase added as a double dosage. When compared to the control, the treatments with xylanase were significantly lower ($p < 0.001$) in underwater pellet swelling measuring, despite the dosage level (Figure 5A, **Paper III**). The same was observed for treatments with the phytase (Figure 5C, **Paper III**).

Different novel ingredients behave differently when pelleted and analyzed under stagnant water for their swelling rate. Pellets containing compressed torula yeast (*Cyberlindnera jadinii*) in the feed and compared to both a positive control (0% yeast) and a negative control (100% yeast) did not defer in swelling within the first 60 seconds (**Paper IV**). However, after 20 minutes of observation time, the pellets with 20% added torula yeast had swollen 1.8 folds and the pellets with 100% yeast had swollen 1.3 folds. Pellets with 100% yeast showed significantly ($p<0.05$) slower swelling when compared to pellets with 20% added yeast for the same observation time. The swelling of the pellets has shown to have a low correlation to the tensile strength of the pellets (experiment 1, **Paper IV**). In experiment 2 (**Paper IV**) the pellet swelling under stagnant water showed to be influenced by the enzymatic hydrolysis. After 40 minutes, the control feed with and without added enzymes was equally swollen. On the contrary, the inclusion of 10% and 20% torula yeast significantly decreased the swelling when compared to the enzymatically treated control feed ($p=0.003$) (Table 3, **Paper IV**). When adding 20% of torula yeast to the feed about a 31% decrease in pellet swelling was observed. Swelling of the feed pellets under stagnant water containing torula yeast showed to be independent of the tensile strength ($R^2 = 0.24$).

Structural differences in the feed pellets caused by yeast and enzymes in **Paper IV** altered the properties of the pellet surface. Such alteration could be described by the diffusion between particles and liquid absorption in the cavities found between particles (Roman-Gutirrez *et al.*, 2003). Defining disintegration of the feed pellets underwater is shown to be a good quality indicator of the usability of feed pellets by aquatic animals, for example, shrimps and abalone, which agrees with Flemming (1995); Obaldo *et al.* (2002); and Bansemer *et al.* (2015).

9.7.2 Surface contact angle

Assessing the surface hydration properties of feed pellets is an important step in understanding the behavior of feed products and their quality control. This is applicable for predicting the storage time in humid conditions and the oil coating process in the downstream feed processing. There are various methods available for this purpose, however, they are not applied in the feed industry but in the food and pharmaceutical industry. For the purpose of this PhD work the surface contact angle measurement (θ), previously used for studying the hydration properties

of wood pellets, was used to evaluate the surface contact angle over time as the visible water or oil absorption degree. Measurements were performed on the top of a compressed flat upper pellet surface (**Paper II, III** and **IV**).

The surface contact measurement accurately captures the absorption rate of a water drop on the pellet surface. This is achieved by observing a decrease in the contact angle of the water drop when protease is introduced to the microalgal biomass (**Paper II**). Protease produced a significant decrease ($p<0.05$) in hydrophilicity when added to the surface of the microalgal pellets. However, the opposite effect was observed when xylanase or lignosulphonate (LS) were added to the microalgal biomass (Fig. 9.5). The surface contact angle measurement showed to be a very precise technique for assessing the surface hydration properties of pelleted materials. Figure 9.5 reveals the variation in contact angle over time for different pelleted mixtures. The microalgal pellets treated with protease exhibited the highest contact angle, indicating lower hydrophilicity. Following this, the pelleted mixture containing xylanase and LS added to the microalgal biomass showed a lower contact angle. The lowest contact angle (i.e. more hydrophilic) during the testing period was observed for the pellets made of pure microalgae. The control pellets revealed the quickest absorption of the sessile water drop followed in order by the mixtures with xylanase and LS. The pellets made with protease had the slowest absorption of the water drop.

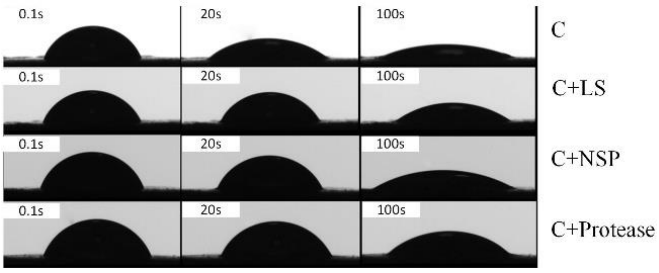

Figure 9.5 - Sessile water drops at the upper microalgal pellet surface during different time intervals. C – control trial; LS – lignosulphonate; NSP – xylanase (**Paper II**)

A good example of showing the benefit of practical usage of the contact angle measurement is also shown in **Paper III** where the type and dosage of added enzymes did not have any influence on the initial contact angle at time zero (T0), either of water or oil drop. Enzyme type and dosage did not influence T0 for the oil contact angle. However, by having the oil drop age for three seconds, xylanase and protease showed increased lipophilicity of the compacted microalgal biomass. There was a statistically significant difference ($p<0.05$) when the enzymatic treatments were compared to the control treatment and treatments with different dosages of mixed protease and phytase. Phytase alone or in combination with other enzymes, independent of the dosage, did not influence contact angle when compared to the control treatment after 1.2 seconds for the water drop or 3 seconds for the oil drop. The same was observed when a mixture of protease and phytase was used or when all enzymes were mixed together and added in various dosages (**Paper III**). Increasing the dosage of all enzymes mixed did not have any influence on the oil contact angle when observed at 3rd second. Differences

of the contact angles could be explained by different interparticle distances in the compacts, which were increased as the oil droplet was aging. These results should not be interpreted as indicating that the enzymes do not affect the contact angle at a higher percentage of enzyme addition. This might be explained by the fact that added enzymes used for this experimental work may be under the quantities that may influence any changes in the contact angle. The differences in the contact angle could be explained by the activation entropy of the protein and the chemical components of the microalgal cell walls at processing temperatures where hydrophobic bonds may become weak (Liu *et al*., 2000). This could be a reason for the particles in compacts to be detached more quickly when oil was placed on them, thus increased lipophilicity was observed.

Also, contact angle measurement showed that different dosages of the investigated yeast did not influence the hydrophilic behaviour of the feed pellets (**Paper IV**, experiment 1). Adding 2.5% and up to 20% torula yeast in the feed did not change the contact angle of the oil at T0 when compared to the control feed. A difference at T0 for oil contact angle was observed between 100% yeast and 0% yeast ($p<0.05$). Pellets containing only yeast were more lipophobic as compared to pellets with no added yeast. The contact angle measurements as a non-conventional methodology showed that time can influence the lipophilicity of the feed pellets containing different dosages of the torula yeast. At 47 seconds the lipophilicity was more pronounced ($p<0.05$) for feed pellets without yeast as compared to feed with 20% yeast and 100% yeast. Similar results for oil contact angle measurements were observed when pellets with no added yeast were compared with 10%, 20%, and 100% yeast at 94 seconds. Pellets with 10%, 20%, and 100% yeast at T0 showed to have pronounced aquaphobic behaviour when compared to control pellets without added yeast. Similar effects were observed at 47 seconds. However, at 94 seconds the water contact angle measurements showed to be significantly lower ($p<0.01$) in the pellets with different dosages of the torula yeast and in the pellets containing only torula yeast.

Paper IV, experiment 2, explains that the contact angle measurements of the water drop placed at the pellet surface and measured every 0.5 seconds for a total measuring time of 2 seconds is not different between all the trials at T0 when having enzymes added. However, after 0.5 seconds the enzymatically treated pellet with 5% yeast had a significantly lower contact angle when compared to the control diet without added enzymes and pellets treated with enzymes containing 20% yeast. Similar observations were seen after 1, 1.5, and 2 seconds of water contact angle measurements (Fig. 9.6). At T0 pellets with 10% and 20% yeast and added enzymes had a significant lipophobic behaviour ($p<0.01$) when compared to other treatments (Fig. 9.7). After 5 seconds the lipophobic behaviour was also observed on the surface of pellets containing 5% yeast and added enzymes. At the dwell time of 10 seconds, this was also seen for enzymatically treated pellets containing 2.5% yeast and 0% yeast. A similar response was observed for the rest of the analytical time, i.e. after 15 and 20 seconds.

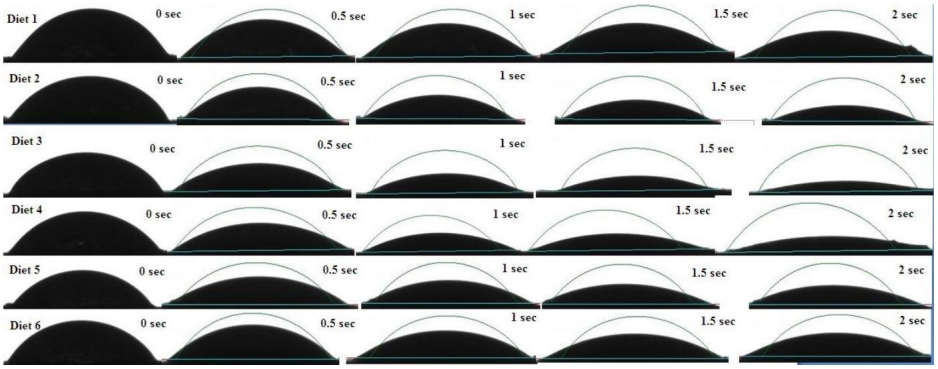

Figure 9.6 - The curved line symbolizes the initial water drop profile (**Paper IV**, experiment 2). Change of contact angle measurement from water drop on the pellet surface at different time intervals for different diets containing enzymes (diet 1, 0% yeast; diet 2, 2.5% yeast; diet 3, 5% yeast; diet 4, 10% yeast; diet 5, 20% yeast; diet 6, 100% yeast)

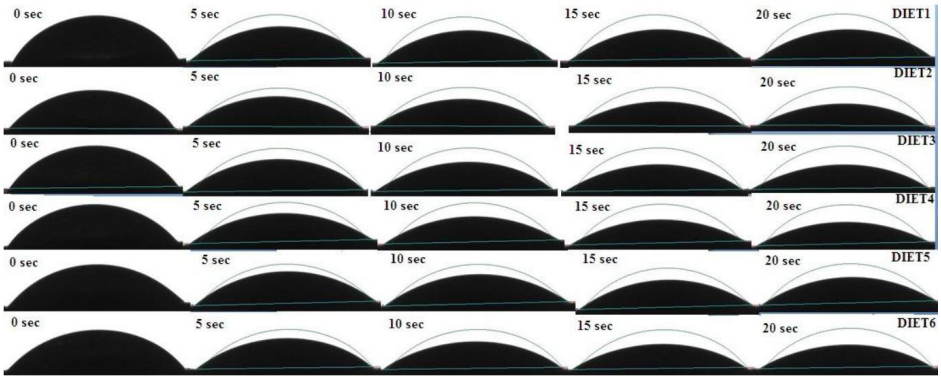

Figure 9.7 – The curved line symbolizes the initial oil drop profile (**Paper IV**, experiment 2). Change of contact angle measurement from oil drop on the pellet surface at different time intervals for different diets containing enzymes (diet 1, 0% yeast; diet 2, 2.5% yeast; diet 3, 5% yeast; diet 4, 10% yeast; diet 5, 20% yeast; diet 6, 100% yeast)

Results related to the interaction of the pellet surface and contact angle of water and oil drop may be explained by the density distribution of the initial powder and further packing of the powder particles in the die. That is well described by Gethin *et al* (2004). The authors explained that different packing of the particles may create two regions in compacted solids with densities differing by 10% during the die-fill. Such variation for various vertical die-fill density distributions with the same total mass filled in the die-hole was explained to be responsible for the difference in the structure and density of the compacted material. Such different final densities may also contribute to differing surface contact angles for both oil and water. The level of physical interaction between the feed pellets and different liquids after being treated

with the enzymes and with different doses of the fishmeal replacing material, yeast, appears to depend on the friction, adhesion, adsorption, and wettability of the surface. This agrees with Yuan and Lee (2013) when other industrial materials are in use as well.

10. Conclusion

Accurate and fast testing techniques should account for the variability within ingredients and the different practices in feed mills. For future process optimization, a good communication skill set based on the scientific premises between technologists and nutritionists is necessary. It is of paramount importance that the structures and functional properties of commercial and novel feed components and their changes with the processing are fully understood. Single-cell organisms, such as microalgae and yeast, show great promise as novel feed ingredients for aquatic animals. However, there is a lack of knowledge regarding the optimal downstream processes for their densification and compaction. This Ph.D. thesis work contributes to filling the gaps in understanding novel feed raw material usage for sustainable feed pelleting in the future (**Paper I**). Also, this Ph.D. work provides insights into the rheologic behavior of single-cell organisms with or without enzymes in an environment with limited water content and pelleted alone or together with other feed raw materials. This scientific work highlights the importance of single-die feed pelleting that emphasizes the need to strike a balance between feed efficiency and the physical quality of feed pellets in small-scale research (**Paper II**). The aim of this work was to improve the creation of high-value feed with better physical quality. This Ph.D. work demonstrates the dose-dependent effects of enzymes on underwater pellet swelling, pressure at incipient flow, and tensile strength (**Paper III**). By adding xylanase and phytase the pressure at incipient flow can be lowered, and thus potentially the reduction of the electrical energy consumption during pelleting can be achieved (**Paper III**). Also, the replacement of fishmeal with torula yeast in the feed increases pressure at incipient flow in the pelleting die, but enzymes can help reduce it (**Paper IV**). It is advised to avoid hydrolysis with enzymes in the feed that will contain 10% and 20% torula yeast to prevent high pressure at incipient flow and possibly excessive electrical energy consumption during pelleting (**Paper IV**). The findings of this work demonstrate that protease and xylanase can contribute to a decrease in the tensile strength of pellets (**Paper III**). The addition of enzymes to feed with 20% torula yeast is recommended to increase the tensile strength of the pellets (**Paper IV**). Additionally, it was observed that lignosulphonates did not enhance pellet strength when added to micro-sized particles derived from microbial organisms (**Paper II**). Pellets with included torula yeast show improved water repellence and lipophobic behavior, while enzymes help decrease underwater pellet swelling and longitudinal surface roughness (**Paper IV**). Protease and xylanase, along with lignosulphonates, were found to decrease the hydrophilicity, underwater swelling, and water absorption rate of the pellets. The measurement of pellet swelling rate through image analysis under stagnant water was identified as a valuable tool for predicting pellet behavior underwater (**Paper II, III** and **IV**). Protease exhibited influencing the lowest swelling and led to fewer hydrophilic pellets, followed by lignosulphonate and xylanase (**Paper II**). Enzymatic treatment should certainly be employed to reduce pellet swelling between 20 to 40 minutes in feed pellets containing 10% and 20% yeast (**Paper IV**). This technique provides insights into the optimal duration that a pellet can maintain its compact form before being consumed by aquatic animals or disintegrating into the water. Further research is necessary to fully explore the potential of underwater pellet swelling measurement and contact angle analysis as methods to optimize their application in the feed manufacturing industry.

The comprehensive approach taken in these studies provides valuable insights that can inform the optimization of pellet production processes, contribute to the development of improved feed formulations, and enhance the quality of pelleted materials in various applications. By embracing these advancements, the feed industry can pave the way for a sustainable future in animal feed production.

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Review article

Future directions of animal feed technology research to meet the challenges of a changing world☆

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ABSTRACT

Feed technology involves the processing of ingredients and the manufacture of animal feeds and is an integral part of animal production systems to provide high quality and nutritious food. The objective is to transform low quality ingredients into higher value feed components, and improve nutrient utilization of compound feeds. Animal feed, therefore, has a social responsibility to contribute to more sustainable food production systems. Further understanding of the structures and functional properties of feed components, their changes with different primary and secondary processing and their conditions, are essential to more accurately meet nutrient requirements of animals. In addition, it will enable a more accurate assessment of overall costs of processing or production with respect to the societal responsibility of feed processing; this may include energy use, carbon footprint, use of water resources and life cycle assessment. Accurate and fast testing technologies should account for the variability within ingredients and the different practices used in the equipment and raw material processing, as well as those in feed mills. Big data will play a pivotal role to model specific aspects of feed manufacturing and could enable the development of a model integrating characteristics of diet ingredients, recipe and processing conditions, whilst optimizing energy consumption, (physical) feed quality and production rate. Collaboration between skilled data scientists, machine experts, feed manufacturing technologists and nutritionists, using advanced data analytics is, therefore, required for future process optimisation. An improved interaction between those responsible for the actual formulation of animal diets, feed

Abbreviations: IFTC, International Feed Technology Congress; IoT, Internet of Things; NIR-S, near infra-red spectrophotometry; PLC/MES, programmable logic computer/manufacturing execution systems; SBM, soybean meal.

☆ Summary of recommendations of the 1st International Feed Technology Conference (IFTC) workshop.

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technologists and mill operators may result in a more constant final feed product quality and the lowest electrical and fossil energy consumption during manufacture, despite inclusion of alternative/substitute ingredients. Lesser known, novel processing techniques may significantly contribute to improve the nutritional value of raw materials and complete feeds. However, only a few techniques may be scalable to economically feasible processes, while others may only be applicable to one animal species and not usable as a general process. In addition, modern feed mills need flexibility and the ability to switch to serve customer wishes and logistics where feed recipes and feed forms are concerned. A major constraint to conduct research in feed processing is the difficulty to acquire attention and funding. More attention should be given to feed additives in processed feed (mainly in pellet form), with research focused on the interaction effects between feed processing conditions, feed components and feed additives. Finally, future feed technologists will require recognized qualifications, possibly to a diploma level. Courses must be successfully completed and include knowledge on smart manufacturing and integrated process control systems. From a feed industry perspective, success of participation in the next industrial feed mill development will be determined by how well staff are prepared and trained.

1. Introduction

Feed formulation and production technology is gaining in importance due to a number of future global opportunities, challenges and threats. Compared to the year 2000, the global demand for animal sourced food is expected to increase by 70 % in 2050 due to growth of the world population, increased income and urbanization (Alexandratos and Bruinsma, 2012; Boland et al., 2013). The world-wide demand for animal feed is expected to increase to 1500 Mton in 2050 with the major growth occurring in Asia and Africa. In addition, animal welfare, environmental pollution minimisation, use of novel ingredients, and the use of ingredients unsuitable for human consumption in relation to efficiency of production, are major challenges facing the feed industry (Babinszky et al., 2019). These challenges are leading to demands for innovation in a number of areas related to animal nutrition including feed technology. Van der Poel and Marchal (2019) argue that the economic opportunities for more advanced diets, both technologically and nutritionally, will increase in the future. The majority of predictions show feed prices will increase in the future and, as technology is independent of raw material feed costs, the economic potential for technology and nutritionally advanced feeds is predicted by the latter authors, to increase.

The 1st International Feed Technology Conference (IFTC) was held in Cologne, Germany in 2019 with the aim to bring together researchers from universities and industry to exchange knowledge and expertise, to share technical and scientific developments in the field of feed technology, and to discuss research results to meet future challenges in animal nutrition. The conference focused on future research directions in feed technology through a workshop for invited experts. The current multi-authored contribution provides an overview of the discussions during this workshop and presents important directions for future technological research in feeds.

2. Objectives for feed technology

Feed technology covers the principles of feed manufacturing where diet ingredients have to undergo processing to manufacture products that meet certain user objectives. Processing ultimately has to have an added benefit and result in an increased feeding value or reduced impact on scarce resources e.g. energy, water, minerals, …. Therefore, feed technology is a means, rather than an objective. However, the current industry focus is no longer only directed at the feed as end-product – we still want to increase the nutritional value of ingredients and end-products – but also on production technology and efficiency since the control of the operations in the factory is emerging with concomitant attention for product quality, mill capacity, environmental impact/emissions control, and production costs. In the feed mill it is expected that ingredients are processed into feeds by grinding, mixing, and agglomeration processes, and optimally adjusted as far as nutritional content and feed form are concerned. This requires reflection on the way we use or optimize certain processes to affect nutrient utilization or feed additive stability, or to inactivate undesired constituents. There are well-known beneficial effects of ingredient and feed processing technologies (van der Poel and Marchal, 2019) and these include:

- Producing a homogeneous mixture of diet ingredients (meal, pellets).
- Decrease negative effects of antinutritional factors in ingredients (trypsin inhibitors, lectins, glucosinolates).
- Increase feed safety (reduction in micro-organisms).
- Increase nutrient digestibility/absorption (better feed efficiency).
- Increase feed intake (less spoilage).
- Find a balance between feed efficiency and animal health (fineness of grind).

The processing of the feed and feed ingredients also involves the design of the feed production line, production methods and technologies, feed product quality control, feed storage and transportation. It is recognized that there is a large diversity in manufacturing lines including flow diagrams, unit operations, equipment, process systems, etc. Even for an operational unit itself, for example particle size reduction, there are different systems that warrant flexibility to have an optimal result in terms of particle sizes/volume (Lyu et al., 2020). Systems for grinding, agglomeration, etc. should be thoroughly evaluated via well-designed studies to

provide information on how to optimize nutrient utilization by animals through choices in the mill process conditions. To meet the demand for new feed ingredients, feed technology has to evaluate every aspect of current techniques to improve the processing and preservation technology for feeds and feed ingredients. Evaluation of our current feed mill processes is not the only means to fulfil the objective for maximising nutrient utilization. Development of new innovative technologies is also imperative. Therefore, scientists currently discuss issues related to feed technology when it comes to basic research, big data, a better communication between technologist and nutritionist and possibilities for training in order to decrease the gap between science and practice. All these issues need attention to support the feed miller to handle future challenges.

3. Importance of feed technology to society

Our current food production systems (plant or animal derived) are, in virtually all cases, associated with the generation of one or multiple co/by-products and waste-streams which are often unfit (or unwanted) for use in human food products. Made possible often only through the application of technology, many of these co/by-products and waste streams are transformed into nutritious animal feed ingredients from which valuable animal-derived food products are produced (e.g. meat, milk, eggs). An essential step in the utilization of food production derived co/by-products and wastes is the appropriate treatment through various technologies. The vastly different co/by-products and waste streams differ greatly in terms of composition (nutrients and antinutrients), microbial load, viscosity, density, flow properties, etc. requiring various technological treatments for optimal transformation to valuable feed ingredients. As such, feed technology already, and more so in the future, makes a highly valuable contribution to the sustainable use of valuable resources and is an integral part of the transition to a more circular agriculture system. Feed technology, therefore, has a social responsibility, by not only contributing to the development of more sustainable food production systems, but more importantly Maslow's fundamental hierarchy of needs (Maslow, 1943).

Feed technology involves the processing of ingredients and manufacture of animal feeds and is an integral part of a sustainable animal production system to provide high quality and nutritious foods. The objective of feed technology is to "upgrade" low quality ingredients to higher value feed components and improve nutrient utilization of compound feeds.

4. Some key basic research in feed technology

The precise and efficient processing of feed ingredients or feed products with optimal available and balanced nutrients depends on a thorough understanding of the molecular structures and function properties (refers to water/oil absorbability, emulsifying ability and stability, solubility, compaction and comminution properties, digestibility *in vitro* and *in vivo*, thermal property of the feed components, their changes with different primary and secondary processing and processing conditions). Taking rapeseed meal as an example, the denaturation temperatures of 12S globulin and 2S albumin in rapeseed protein by differential scanning calorimetry (DSC) were 81 and 60℃, respectively (Zhou et al., 2013), and moderate processing conditions (15 min, 80℃) can improve the oil absorbency, emulsifiability and protein digestibility of rapeseed protein, but have no obvious improvement in protein solubility. The different fractions (particle size: 212~425, 150~212 and 106~150 μm) of finely ground, low temperature defatted rapeseed meal with hulls have different functional properties, the emulsifiability, emulsifying stability and protein digestibility of samples obviously increased ($P < 0.01$) with the particle size reducing (Feng et al., 2015). Pulverizing the low temperature defatted rapeseed meal with hulls into to four different geometric mean diameters (13.2, 81.7, 138.9 and 176.9 μm) and treatment at four heating temperature (60, 80, 100 and 120℃) for 4 different heating times (30, 60, 90 and 120 min), showed that the influence of factors on protein solubility, water absorbability, oil absorbability was "particle size > heating temperature > heating time". But for protein digestibility, the sequence of factors was "particle size > heating time > heating temperature" (Feng et al., 2016).

In feed ingredients, the molecular structure especially the three dimensional molecular structure of single chemical components, such as lysine, protein, starch, dietary fibre, etc., determines its basic functional property and availability to animals. Important is that all the single chemical components exist in a more complicated matrix and they usually combine or link with other chemical compositions, thereby changing their three dimension molecular structures and their functional properties and availability to animals. Processes such as grinding, pulverizing, wet or dry conditioning, roasting, pelleting, extrusion, etc. will further change their three dimensional molecular structure, functional properties and availability to animals. Future research should include the study of the three dimensional molecular structure and functional properties of protein, starch and fibre components, etc. in feed ingredients and feed products, their changes and changing mechanisms with processing conditions. Through this research, practical processing recommendations can be developed to obtain the optimal functional properties and availability of feed protein, starch and fibre components, and furthermore the functional properties and availability of the feed ingredient and feed products.

Accurate and rapid testing technologies of the molecular structures and functional properties of the feed components are necessary. Synchrotron Infrared Microspectroscopy and Diffuse Reflectance Infrared Fourier Transform Molecular Spectroscopy can be used to detect some processing-induced molecular structure changes in protein, lipid and carbohydrates related-functional groups within intact tissues (Yu, 2012), but research on this issue should be strengthened. Fast testing technologies for functional properties of feed components, ingredient and products are also needed.

5. Nutrient and processing variability

The feed mill industry is conscious of the need for high nutritional quality of animal feeds. To achieve this, the variability in dietary ingredients and those related to the feed mill processes, should be carefully controlled.

Feed ingredients may vary considerably in their nutrient content, not only across ingredients, but also within a single ingredient. Soybean meal, for example, receives a lot of attention in which the ileal digestibility of amino acids (apparent or standardised) is an important target criterion in addition to crude protein/amino acid levels. There are currently only a few studies that document variation in amino acid digestibility between different soybean meals that also explain this variation (Table 1; J. Coma, 2015; personal communication).

Reasons for this variability are the variations as to plant genotypes, environmental conditions during growth, harvest/storage conditions, and also due to the final processes that affect the nutrient composition of the co/by-product. Without a complete, accurate and timely nutrient analysis of feed ingredients, feed formulations tend to be over-formulated to meet the animals requirement to account for this variability in nutrient levels of ingredients. The latter ensures that growth rates of the animals are achieved in case low nutrient content ingredients are used in feed formulation. This approach increases ration and production costs and contributes to environmental pollution. The quantification and understanding of the variability in protein and ileal amino acid levels (total and digestible), as well as other nutrients, remains important and requires further scientific and practical attention.

Process variability is the result of inaccuracies in processing from e.g. weighing systems (deviating ingredient/additive levels), sampling techniques to unknown effects of process equipment/conditions on nutrient utilization (changed digestibility values). Often, sampling techniques/protocols in regular production facilities are sub-optimal, thereby affecting analysed results as the feed sample is the 'foundation of feed composition data' (Weiss and St-Pierre, 2014). Using proper sampling and analytical techniques is still a major challenge in feed mills with investments in on-line quality control systems such as NIR-S providing potential solutions to increase the accuracy in feed composition data. Having an accurate estimation of the nutrient content of feed ingredients (total as well as digestible) will result in more effective feed formulations. In relation to effects of process equipment/conditions on nutrient utilization, more knowledge on the effects of ingredient/feed processing on nutrient utilization should be developed. Salazar Villanea et al. (2017) showed that an increased rapeseed meal toasting time after oil extraction leads to a severely decreased level of total and reactive lysine, and reduced performance of growing pigs. It is generally known that the conditions during important processes such as grinding, extrusion and steam treatment can markedly affect the nutritional value of the product. Basic research, however, has shown that the physical changes in the proteins (denaturation) progress more rapidly than the chemical changes such as Maillard reactions. The research of Salazar Villanea (2017) showed an increased protein and lysine digestibility in toasted rapeseed meal in pigs after pelleting, as well as after extrusion, allowing a broader scope for rapeseed meal to be used in (pelleted or extruded) pig feed formulations. It also shows the importance of determining the interaction between ingredient processing (e.g. toasting of soybean and rapeseed) and processing of the feed (e.g. pelleting, extrusion, drying).

Results of research into feed and ingredient processing are expected to have a more profound role in the future with respect to ingredient/nutrient constraints in diet formulation (linear programming). Knowing the precise effects of processing on inclusion levels of ingredients (e.g. pulses, oilseed meals) in feeds will allow more precise feed formulation by having accurate constraints. Higher inclusion levels can be used in feeds that will be pelleted when precise effects of processing are known. In addition, more accurate constraints can also yield improved feed quality in terms of meeting nutrient specification by understanding the influence of processing effects on nutrient utilization when it comes to different varieties of ingredients (e.g. soybean meal from different countries, Table 1). For this type of research, a more coordinated approach should be considered regarding the quantification and understanding of the variability of the different processes used in the equipment and ingredient processing industry (primary processing), as well as those in the feed mill (secondary processing).

6. Big data for development of feed technology processes

Unlike other fast developing industries such as information technologies or the automotive industry, etc., feed manufacturing can be characterised, at first glance, to be a traditionally-oriented industry. This is in part driven by consumers who are not open to innovations in the agro-food chain, having preferred concepts on what animals should eat, and how that might affect food products and the human diet in general. In part, it is influenced by the high volume based production approach of feed manufacturing. As feed mills are increasing in capacity at the same time as margins remain low, investment costs in advanced equipment is difficult to economically justify, since it negatively influences the price of the product. Costs of feed already contribute between 60–70 % to the total livestock production costs (Coffey et al., 2016).

Although the industry seems to be rather traditional as their principal operations are known and have been used for decades, feed

Table 1
Variability in crude protein and lysine content, lysine to crude protein ration, ileal NIR-S lysine digestibility and digestible lysine content of different soybean meals for pigs (J. Coma, 2015; Personal communication).

	Crude protein g/kg SBM	Lysine/CP g/100g	Lysine SID[a] g/100g	Digestible lysine g/kg SBM	
Average	461.2	6.1	88.0	24.8	
SD	12	0.1	1.3		
Minimum	449.2	6.0	86.7	23.4	
Maximum	473.2	6.2	89.3	26.2	Δ = 2.8

[a] NIR-S standardized ileal digestibility (SID) lysine for pigs; CP, crude protein; SD, standard deviation; n = 7265.

processing equipment is under constant development in order to enable more efficient production of safe and nutritious feeds. The first "computerized feed plant" became operational in 1975. However, seeing the capabilities of equipment in the 70′s compared to today, it is clear that the term "computerized" is no longer comparable to today's standards. Starting from simple machines where adjustments were made manually to recent developments where fully automated processes can be run from a remote location (Shofield, 2005), the industry has made great strides in efficiency and flexibility, a development which will continue in the future.

Modern feed manufacturing equipment has possibilities to vary a wide range of different parameters to obtain key performance indicators at the target level. For example, hammer mills can be adjusted remotely to an optimal rotational speed, or automatically change sieves if variation in throughput and energy consumption is required. Also, variation of the roll speed of the pellet press would be an indicator of slip between roller and die, potentially indicating pellet press blockage, requiring a change in operating parameters. What previously was the task of the skilled process operator, now becomes the task of the central control loop system of the feed plant unit operation (Guster, 2019).

In the Internet of Things (IoT) era, signals of each of the machines in the line are collected. Even more, properties of raw materials, such as chemical composition, particle size, or bulk density are continuously monitored by in-line sensors, such as NIR-S, resonance technologies, vision cameras, etc. The data collected from these sensors are in turn matched with the data collected from the machines in the processing line. In addition, calibration of sensors (e.g. NIR-S) poses additional challenges as changes in sensor settings require either a reparameterization of the predictive models used, or a recalculation of stored data for these specific sensors to match an existing model parameterization.

Algorithms are able to determine relationships between process parameters and to optimize the feed manufacturing process in real time. Examples of the use of artificial neural networks within the feed industry are provided by Pathumnakul et al. (2009); Sudha et al. (2016) and Ittiphalin et al. (2017). However, these models only optimize part of the feed production process. Pathumnakul et al. (2009) modelled production rate and dust levels in the pellet as a function of process parameters and raw material levels. Sudha et al. (2016) examined the optimization of production rate as a function of process parameters. Ittiphalin et al. (2017) investigated the total amount of fat addition and the distribution of added fat over the main mixer or coated on the pellet. These studies show that attempts to model certain aspects of feed manufacturing can be successfully implemented. However, an overall model integrating raw material characteristics, recipe and process condition, whilst optimizing for energy consumption, (physical) feed quality and production rate has, to date, not been developed.

If the target values of responses are set, either by company experts or by customers, continuous build-up of company databases would occur. These databases would serve as a knowledge base for a specific company and would continuously expand and would not be compromised when skilled professionals would leave the feed mill company, as currently is the case. As the databases are established for single production sites, those companies having the possibility to match data from different production sites can try to find correlations between parameters and learn from the integration with the knowledge of other sites. Such companies would have an advantage to increase their knowledge and ultimately improve efficiency. Although there might be differences within the process line layouts, it is to be assumed that a single company would have similar targets for outputs controlled at different production sites. The company experts would have to deal with these data and convert it to general company knowledge.

But what if different companies would be able to combine their databases? Exchange of databases would increase general knowledge, and as the number of feed mill companies willing to share the knowledge would increase, the databases would be more extensive and information would yield knowledge in common for all companies involved. In that case, personal experience would become joint experience. A main goal is to develop and share innovative bonds between data storages dedicated to high-throughput delivery of big data generation and analysis. Computer databases and data management and analysis facilities would be required for handling the huge amount of data relevant to animal feed production and for simplifying the localization, which would enable the extraction and the analysis of relevant information (ATF, 2016).

There is a common interest to have access to good quality public data, but there are several challenges to be overcome. Through such an approach the above-mentioned social responsibility statement regarding feed technology could be more rapidly and effectively implemented used by companies and advance their sustainability reporting and marketing.

6.1. How to deal with big data; what is the target? What do we want to optimize for?

As feed companies might have similar general interests, which are to produce and sell large quantities of nutritious and safe feed, with minimal productions costs and maximal profits, it is to be expected that big data would be used in a similar manner. However, development of joint networks would aid in the collaboration between many different stakeholders having different roles and targets in the data value chain (Wolfert et al., 2017). Targets of the feed producers related to outputs such as throughput, specific energy consumption, physical quality of pellets, etc. may depend on the specific manufacturer. On the other hand, the interest of farmers is to have feed with high digestibility of nutrients, and these values should also be considered when the data are interpreted. Yet, collection of the nutritional parameters would require additional resources, without certainty that data collected would be possible to be used for different process environments. Therefore, big data applications would not be strictly about production of feed but may play a major role in improving the efficiency of the entire supply chain, with focus on food security, safety and sustainability (Gilpin, 2015).

6.2. Can companies share data?

It is to be expected that companies would initially be reluctant to provide "outsiders" access to their internal data. They would want to be in control of who can access and use their data. To have benefits of joint databases, business models for sharing of data and open

data sources should be developed. Also, recognition of ownership of data is crucial, and portals to facilitate exchange of data are a prerequisite (ATF, 2016).

6.3. Feed manufacturing evaluation systems

Additional problems might occur when interpreting data collected by different sensors and logging systems available in the feed processing environment. For the same output, such as temperature or pressure, the data collected might vary depending on the type of sensor, as well as accuracy and position of the sensor. Likewise, calibration of equipment (e.g. NIR-S) contains data of local importance, since they are developed for specific feedstuffs at specific locations, with specific equipment used. As the goal is to keep specific output in the required range to have constant quality of the product, it would be challenging to have general solutions implemented in the same manner at different locations, since the observed values would differ depending on the measuring equipment used. Effects on product quality would also differ. Here, a unifying framework would permit identification of how certain processing characteristics are calculated. In addition, the availability of multiple sensors permits additional checks and balances on the quality of the data, e.g. if both a steam flow measurement system and a temperature sensor are mounted in the conditioner, both sets of measurements have a certain relationship to one another. If, within certain margins of error, this relationship is not maintained (which can be calculated and checked at the PLC/MES level in real time), the operator could be notified on the quality of the incoming data.

In addition to checks and balances on the quality of the incoming data, a consistent vocabulary should be developed to clarify the meaning of certain processing variables, e.g. production rate (ton/h) is an important parameter for all feed manufacturers. This, however, can be interpreted in different ways:

- Net production rate: the number/weightof pellets produced per time unit (e.g. week, month year) for a given pellet press or line: a figure of most interest for economic reasons.
- Steady state rate: rate of pellets produced per pelletizer when running at steady state, without start up or stopping time included for that specific batch or run. This rate is higher than the calculated net production rate or batch/run production rate. Of interest for optimization purposes; most of the time this parameter is targeted by the process operator.
- Batch or run (multiple batches of the same feed) production rate: production rate including start up and stop time for a given batch or run. During these time periods the production rate is increasing to the steady state rate (start-up) or falling to zero (stop). This production rate is lower than the steady state rate and higher than the net production rate.
- Rate including recycle: in pellet line designs where sieved off material is recycled to the feed mash bin (or more preferably to the dosing screw), additional load is placed on the pelletizer to pelletize fresh feed mash plus a certain percentage of fines returned. Since in most factories the amount of recycle is not measured, this introduces an additional reduction in net production rate due to re-pelleting of fines into pellets and increases the amount of electrical energy used in pelleting. For example, for a 10 ton batch with a recycle percentage of 10 %, an additional 1 ton of feed needs to be pelletized by the press, reducing the net production capacity. It does not affect the steady state rate and increases the batch or run rate (since start and stop time are a smaller fraction of the total pelleting time). In addition, steam and electric energy are used to pelletize this additional recycle material.

Such standardised vocabulary should be developed for all process and systems parameters.

6.4. Do we have expert knowledge to interpret all these data?

Data bases might be compiled from a large amounts of unstructured and heterogeneous data which requires interaction between skilled data scientists, machine experts, feed manufacturing technologists and nutritionists using advanced data analytics. Without a doubt, ‘big data’ analysists will become an important asset for each feed manufacturer. To make full use of this potential it is necessary to develop additional vocabulary to precisely identify targets for the feed manufacturer in terms of nutritional and physical quality of the feed at optimal processing conditions. ‘Big data’, without expert knowledge, is meaningless. In addition, ‘big data’ is associated with approaches such as machine learning and artificial intelligence and these two areas may need to be employed in the future to facilitate data interpretation.

7. New technologies for a different (regions) and a changing world

It is well known that the rising demand for valuable, animal-derived food products can only be met by sustainable agriculture systems. The future of livestock production to supply animal-derived food products, and the contribution to supply quality proteins for human well-being, depends on the use or application of raw ingredients to supply feeds. It is vital that animal and aquatic feed manufacturers use sustainable, secured and safe resources. The feed industry relies heavily on co/by-products and wastes of the food industry, but also on common ingredients that directly compete with those used for the production of human food products. This is a key reason why animal and aquatic feed production is subject to volatile markets. According to the reports developed by the Food and Agriculture Organization of the United Nations (FAO, 2019), the global commodity price index rose by 50 %. Ingredient prices for soybean meal, fish meal, maize, wheat and oils for aquatic feeds rose by 67, 55, 284, 180 and 250 %, respectively, over the last decade. This global price-increase-phenomenon affect small farmers disproportionately, and potentially have a negative impact on global poverty issues. Such uncertainties can be reduced by finding and developing alternative feed ingredients, or by the development of suitable techniques to recycle more of the food wastes, and transform these into valuable ingredients for the aquatic and land-based

animal feeds. Forest and marine biomasses can also be used to obtain valuable carbohydrates, proteins and fats for animal diets. Creating value through the approach of a circular economy will always dependent on profitability within a world economy.

7.1. Alternative ingredients

To refer to some ingredients as 'alternatives', detailed (scientific) investigations must approve them to be safe for specific animal species. A number of aspects inherent to the production, composition and processing of 'alternatives' are important (van Krimpen and Hendriks, 2019). From a nutritional perspective, the composition and quality, nutrient digestibility/availability, presence/absence of nutritionally active factors, palatability and concentrations of contaminants are important. From a feed manufacturer's perspective, criteria such as availability of supply, potential inclusion rates, stability, handling and storage and effect on pellet quality and final feed are key. Moreover, 'alternative' ingredients should preferably not increase the energy consumption during feed manufacturing unless inclusion reduces feed costs to compensate. For 'alternatives' to be cost-effectively applied as an animal feed ingredient, variability in composition and quality, and its effect on the quality of the final product (meat, milk, eggs), should also be considered. As feed manufacturing is a continuous business, a regular supply of large quantities throughout the year has to be guaranteed or – alternatively – is part of the flexibility at the mill level to be able to take advantage of its seasonable availability.

The necessity for finding alternative ingredients for animal feeds has already resulted in a large number of academic research articles. For example, inclusion of single-cell organisms to replace fish oil (Naylor et al., 2009) or to replace fish meal has been studied. Good substitutes for fish meal may also come from the bacterial meal (Øverland et al., 2006), yeast (Grammes et al., 2013; Vidakovic et al., 2016, 2019) or microalgae (Taelman et al., 2013; Grammes et al., 2013). Single cell organisms, like microalgae, are ingredients that could also be used in food products (Batista et al., 2012) and animal feed products (Patil et al., 2005). Some single-cell organisms possess useful immune and health stimulating benefits of the aquatic organisms (Reveco et al., 2013), which may be necessary to ensure products are economically competitive. The importance of finding new/alternative feed ingredients will become increasingly important due to the increasing demand for animal feeds, coupled with the increased use of human edible feed ingredients in food products (Boland et al., 2012).

7.1.1. Bacterial meal

Bacterial proteins have proven to be effective nutrient sources for monogastric animals, and have significant potential due to their fast growth on substrates, independent of climate conditions, water resources and soil. Optimal chemical composition of bacterial proteins and their effect on nutrient digestibility, metabolism and growth performance in broilers, pigs, mink (*Mustela vision*), foxes (*Alopex lagopus*), Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and Atlantic halibut (*Hippoglossus hippoglossus*) have been reported (Øverland et al., 2010). In addition to a good nutritional profile, the dietary inclusion of bacterial meal can prevent inflammatory processes/enteritis, induced by solvent extracted soybean meal found in the diets of Atlantic salmon (Romerheim et al., 2011). Also, feeding bacterial protein reduces levels of lipid oxidation in chicken meat during frozen storage without consequences on sensory parameters linked to flavor of the meat (Øverland et al., 2011). The bacteria production process is, however, still rather expensive when compared to other single cell resources due to its growth dependence on natural gas and to the gas-price.

7.1.2. Yeast

Usage of yeast as a protein source was investigated in different fish species. The research results show that brewer's yeast has a very high protein digestibility and a high gross energy level in the red claw crayfish (Pavasovic et al., 2007). In Atlantic salmon diets, several yeast species (*Candida utilis*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*) showed that there was no difference when fed in terms of feed conversion, specific growth rate and final growth rate, compared to fish meal (Øverland et al., 2013). When *S. cerevisiae* was compared to other yeast-based diets (*C. utilis* and *K. marxianus*), it was observed that fish fed *S. cerevisiae*-containing diets had higher daily feed intake. However, fish fed with a diet based on *S. cerevisiae* had decreased retention of nitrogen and energy, while fish fed the *C. utilis* diet had increased nitrogen retention compared with those fed the fish meal diet. In general, feeding the *S. cerevisiae* diets resulted in a lower digestibility of crude protein, amino acids, and energy compared with fish meal, *C. utilis* and *K. marxianus* containing diets. The intact cell wall of S. *cerevisiae* was shown to have a negative impact on the apparent gross energy and amino acids digestibility in Artic charr (*Salvelinus alpinus*) when compared to diets without cell walls (Langeland et al., 2014). Another fish species, rainbow trout (*Oncorhynchus mykiss*), fed *S. cerevisiae* as a fish meal replacer, showed similar blood and plasma amino acid profiles as the fish fed with fish meal, without differences in acute stress response (Huyben et al., 2017). As such, yeasts seem to be an attractive alternative protein source in fish feeds.

7.1.3. Microalgae

Microalgae have high levels of protein, carbohydrates, lipids and antioxidants, and as such stand out as a promising novel ingredient (Pulz and Gross, 2004; Teuling, 2018). Microalgae might also reduce the ecological impact of the current intensive use of fish meal for feed manufacturing (Muller-Fuega, 2000) making microalgal biomass use in aquaculture an environmentally sustainable option (Taelman et al., 2013).

When single-cell biomass becomes available at a suitable and non-volatile price, its utilization in the aquatic feed industry would be noticeable. Chlorella and spirulina microalgae are well known for their protein content. Furthermore, the microalgae *Desmodesmus subspicatus* is known for its large lipid storage (Xin et al., 2010) and may be a suitable fish oil replacement. Marine microalgae are primary producers of omega-3 fatty acids, EPA and DHA. Thus, instead of using processed fish oil *Desmodesmus subspicatus* or other marine microalgae could be used as the EPA/DHA source in the animal feed.

7.2. Novel technologies

For the use of unicellular organisms as feed ingredients, disruption of the cell wall before mixing the material with other ingredients is critical for an effective upstream process. A range of cell wall rupture techniques have been tested with overall positive effects on yield. High pressure homogenisation (Halim et al., 2012; Olmstead et al., 2013; Samarasinghe et al., 2012), as a technique used for disruption of the cell walls, using a pressure gradient and increasing the turbulence within the product, produces strong shearing forces in the compressed suspensions. Thereafter, fast depressurization of such suspensions forces the cell membranes to rupture and release intracellular contents. Ultrasonication (Keris-Sen et al., 2014) is a process where sound energy agitates particles of a sample where frequencies higher than 20 kHz may result in the extraction of bioactive compounds from plants, microalgae and seaweeds. Microwave heating (Balasubramanian et al., 2011) involves electromagnetic waves and heat transfer within treated material, which is produced by a fast change between electric and magnetic fields, and where dipolar molecules of water oscillate fast. Ball-milling (Balasundaram et al., 2012) creates the crushing of the material found in between non-elastic balls found in a sealed container. Such techniques can locally generate high pressure and thereby influence a crushing of the cell walls. Osmotic shock (Lee et al., 2012) engages a fast shift between a high osmotic pressure and a low osmotic pressure solution by diluting samples with, for example, water. In such a rapid osmotic shift, the cell membranes may weaken.

The economic feasibility of these novel processing methods are yet to be explored. These processing techniques may significantly contribute to rupturing the cell wall, however, only a few of these techniques may be up-scalable to processes economically feasible for application at a large scale. Any technology, to be economically suitable, needs to be able to process for example unicellular biomass equally with low and high-water content as well as with low or high densities and viscosities. Also, such technologies need to be remarkably energetically effective, with a short processing time, and with minimal end-product degradation.

8. Technology attuned to different animal species - Encapsulation technology for targeted release of in-feed bio-actives in the gastrointestinal tract

In general, animal feed production processes single feed ingredients or compound mixtures to improve nutrient availability and to a physical form to efficiently meet the nutritional requirements of animals for optimal performance and/or economic returns. The effects of the applied processes on macro- and micronutrient availability depend on the production parameters and processing equipment utilized in the mill. A number of feed processing steps can influence nutrient availability, including raw material grinding (particle size), mash conditioning (temperature, moisture content, retention time), pelleting (die geometry), extrusion (temperature, pressure, shearing, moisture content, retention time) and cooling/drying systems. However, thermal processes with the most thoroughly documented effects on the physical and/or chemical changes of nutrients are conditioning, pelleting and/or extrusion. Conditioning is the process of the addition of steam (and water) in mash material which results in its heating and hydration, and consequently partial cooking. Pelleting is the process of converting the conditioned mash feed into granulated products by means of mechanical pressing throughout the die, whereas binding is facilitated by moisture and heat. Extrusion is a continuous process by which moistened materials are plasticized and cooked by a combination of moisture, pressure, temperature and mechanical shear, resulting in more intensive physical and chemical changes compared to the pelleting process (Riaz and Rokey, 2012; Rojas et al., 2016).

Although requiring additional investments, and increasing operational costs, the benefits of thermal processes in livestock nutrition are numerous. For example, the pelleting process has positive effects on mixture stability, volume reduction, avoidance of selection of specific ingredients by the animal, improvement of feed intake and feed conversion, increase in product value, improvement of sensory properties, and improvement of hygienic status of the feed. The pelleting process, including steam conditioning, usually lasts less than 1 min, but can be also longer with an added hygiene step; temperature typically ranges between 60 °C and 90 °C, while the moisture content does not generally exceed 170 g/kg. Under these conditions, effects on macro-nutrients are generally limited. However, depending on the feed processing conditions applied, concentration and activity of thermolabile micro-ingredients can be significantly reduced in final feeds. Extrusion processing exposes the mash to more extreme parameters than the pelleting process and is used to manipulate physical qualities of feeds, alteration of texture, starch gelatinization, protein denaturation, destruction of microorganisms and toxic compounds, etc. While the retention time in the extruder is generally short (approx. 30 s), extrusion applies more severe conditions, such as higher temperature (typically between 90 and 150 °C), and higher moisture content (up to approx. 300 g/kg), and high shear. Under these conditions, negative effects on thermolabile additives are more pronounced (Kim et al., 2016).

Some components, such as vitamins, carotenoids and other bioactive compounds (e.g. essential oils) are included in very small amounts (e.g. µg to mg/kg) but contribute significantly to overall diet costs. Vitamins are essential nutrient cofactors in all biological functions. Therefore, depletion of such valuable components can have severe effects on animal health and performance. Adjusting feed processing parameters to lower temperatures to limit destruction of vitamins might also compromise other beneficial effects of thermal processes. Therefore, thermolabile components are typically added in protected (encapsulated) form, or via post pelleting liquid application to avoid degradation during the feed manufacturing process. Coating aids used in the encapsulation process can consist of starch, polysaccharides, proteins, gums, resins, lipids/fatty acids, or a combination of these. Furthermore, different physical methods used in the encapsulation process of feed ingredients, such as spray drying, freeze-drying, emulsion, entrapment, prilling, chelation, coacervation, fluidized bed-coating and extrusion, can also affect the physical and functional properties of the end products. Encapsulation provides protection for micro-ingredients by coating them with different coating aids and with different processing techniques to improve their resistance not only to high temperatures, but also to light, oxygen, pH and humidity. In some cases, encapsulation is also used to prevent flashing off of thermolabile compounds (e.g. essential oils), or to prevent odour issues of some bioactives (e.g. short chain fatty acids), and to modify physical properties (e.g. sinking vs. floating particles within the rumen). While

encapsulation provides many potential added benefits, the encapsulation processes increase the cost of bio-actives relative to unprotected forms (McClements, 2018; Shahidi and Han, 1993).

In ruminants, additional protection may be necessary from a physiological perspective given the ability of the rumen microbes to degrade/utilize unprotected bio-actives, especially when the target area of the digestive tract is post-ruminal. In this case, different physico-chemical approaches may be required. In some instances, raw materials (e.g. proteins) must be pre-treated to prevent ruminal degradation (e.g. via chemical treatment or encapsulation) to deliver higher amounts of rumen by-pass protein for milk production.

Encapsulation technologies offer new possibilities to tailor feed additives to support targeted biochemical pathways. By using specialized coatings/processing methods within the encapsulation process, it is possible to deliver additives to a target location in the digestive tract (e.g. rumen by-pass, target release near caeca), which can positively affect animal performance, health and welfare status. Products, which may supply by-pass fats/amino acids, essential oils, buffers, prebiotics, probiotics, among others, can be designed to release bio-actives at desired locations to exert their intended functions, with the potential of blended products with multiple target-release bio-actives. Since encapsulation increases the cost of additives, it is principally micro-ingredients with high raw material costs that are protected. Macro feed ingredients can be separately thermally/chemically treated (e.g. by-pass proteins) but are usually not subjected to "expensive processes", such as encapsulation, before being incorporated in the feed (Celi et al., 2017).

In this respect, animal feed can be considered as a mixture of ingredients, out of which, some of the ingredients are able to release their activity or to be utilized at precise locations, while for others there is a balance between the needs of the production process, animal requirements, and production costs. But what if there would be possibilities to adapt feed production processes to reflect animal needs? By following this "inside-outside approach" individual components of the compound mixture could be delivered to a target location and digested/absorbed optimally, tailored to the species in question and/or to the functional role of the product. To achieve an "encapsulated pellet", each of the ingredients, or the groups of ingredients, should be pre-processed separately before being combined with other ingredients. With such an approach, nutritionists, veterinarians, and other experts could provide clear inputs to animal feed producers on the precise animal needs. Also, as livestock is part of the circular economy, tailoring raw material and feed production processes could address not only animal performance and health, but also emission mitigation, sustainable nutrition, responsible use of antimicrobials, to name a few. However, the complexity of the processes and optimization parameters would also increase production costs. It will be interesting to see if, by applying novel encapsulation and feed processing strategies, the gap can be closed between actual performance relative to the genetic potential of livestock.

9. Flexibility in current feed mills

In discussing the manufacture of animal feed, three main compound feed quality aspects can be distinguished: product quality, food safety and safety for the environment (Klein, 2015). For the manufacture of animal diets, the basic processes such as grinding, mixing and pelleting are widely used. In mill design, however, there is a constant change in that specifications of animal feeds are regularly tightened in view of consumer and social demands (sustainability; availability of raw materials; new ingredients) and those imposed by legislation (nutritional; environmental). Therefore, new ingredients and process technologies are becoming more important. Further developments are the on-farm processing in some countries where farmers manufacture part of their own animal feeds and/or purchase supplementary compound feeds to their own grown cereals (Klein, 2015; van der Poel and Marchal, 2019).

All these changes are paralleled by new developments (e.g. big data) in the process, automation/use of sensors and the rapid methods (near infra-red analysis; NIR-S) for nutrient analysis and process effects. Near infrared developments are generally important when the number and accuracy of nutrient analysis is increasing. It can be used for in-line analysis of ingredients, to optimize moisture level and to reduce nutrient variation. The use of NIR-S for process optimization to meet nutritional and feed safety objectives is currently being examined and will be a very important factor in the future of feed manufacturing.

In general, more flexibility at the production location is desirable as well as development of innovations in compound feed manufacturing. The latter requires more disruptive thinking rather than thinking along well-known paths. Apart from our modern-day feed mills (diet processing by grinding, mixing and pelleting as the main processes), more emphasis is seen nowadays towards technologies where flexibility in processes is achieved to be able to deliver different diets/feed forms to the farmer. There is often tension between the routinely used diet ingredients and the (high capital) technological infrastructure that dictate the ways we manufacture animal feeds.

In thinking somewhat 'out of the box', one can ask if the current feed mill will still exist in the future, and if the routine operations of grinding, mixing, pelleting and the use of micro-ingredients will still be commonplace. The 'ambitious' or 'technologically advanced' farmer may also have operations such as grinding and mixing on farm with limited investments. Pelleting is not essential when considering feed form/transportation, although the positive effects of pelleting on the feeding value are important. Ingredient traders can be approached by farmers, whereas consultants can help in feed formulation. Since purchasing non-local diet ingredients is not a main task of the feed miller, thinking out of the box then means that the added value of the feed mill should come from upgrading separate diet ingredients by heat, pressure, shear, enzyme technology or other treatments. In light of the latter, one can argue that a more flexible feed mill from the point of view of technological treatment of ingredients is necessary. Some feed mills are designed to be flexible in ingredient processing. Ingredient processing is routinely carried out in specialized industries just because the process conditions used in these treatments are not found in the routine feed mill. Developments are, however, that some companies include these primary processes in their feed mill, for example corn flaking to affect starch fermentation/digestion in ruminants.

Early ideas for a novel way to manufacture compound feeds were described as a concept known as 'cafeteria diets' for pigs (Ammann, 1989). In this concept, separate raw materials (cereals, pulses, beet pulp, pre-mixtures) are all pelleted where a final compound feed is established by just mixing the 'ingredient pellets' to a final feed. In this concept, the grinding and pelleting processes

can be attuned to individual diet ingredients, with advantages in the choice of their process conditions. Research showed that for pigs of 30 kg, no negative effects could be seen in terms of performance (van der Poel et al., 1997). Practical problems, however, can be the presence of different pellet sizes (selection by the animal), different pellet densities, and the inclusion of fat, bio-actives (e.g. enzymes, vitamins) or a pre-mixture that have to be solved.

As mentioned before, the feed industry is rather traditional since the principal operations have been known and used for decades. Feed processing equipment is under continuous improvement to enable a more efficient production of better end-products. If flexibility in feed forms is desired, another mill design is required in a way that all kinds of feed forms such as pellets, meal, expandates, muesli, kibbles, etc., can be manufactured. In such a case, feed mill design and choice of equipment go hand in hand relative to the desired quality and costs of the process. For this reason, different pre-compaction methods (e.g. expanders, BOA compactor, double pelleting) in the pelleting process are used. For feeds where hygienic requirements are high, e.g. poultry diets, long-term conditioners operating at higher temperatures are used prior to pelleting. In these cases, a feed mill has to be able to switch to serve customer wishes and logistics.

Feed mills may also be flexible in their compound end-products and may produce compound feeds supplementary to basic ingredients that are available on farm. Farmers growing their own cereals, or that have access to other ingredients can, by purchasing supplementary feeds and home mixing, produce feeds themselves. The supplementary compound feeds will have a higher nutrient density compared to cereals as the latter are a lower nutrient dense feed ingredient compared to the nutrient requirement of the animal. In order to produce supplementary feeds, feed mills should have the flexibility to produce, among others, this type of feed. If the farmer has its own cereal production, the complementary formulation has no cereal component and the compound feed should involve the production of a supplementary high-fat formulation for which the techniques in the feed mill may not be available, or insufficiently equipped. Special equipment in some cases may be required such as sprayers or vacuum coaters or the problem has to be solved by the application of lipid-rich ingredients, pellet binders or a high pressure pelleting process; the use of post-pelleting devices is employed to include higher fat levels that can be paralleled by the addition of heat-sensitive feed additives (Pierce et al., 2003).

A similar case can be made for the application of wet co-products, especially meant for the feeding of pigs and cattle. Co/by-products from the fermentation of wheat/corn to produce ethanol can be used as long as the ecological foot-print of this kind of co-products is not too high, and investments are made in liquid feeding systems for pigs, or a feed mixing device for roughage with supplements on dairy/cattle farm. Quality control of the liquids/high moisture products is essential, as is control of pellet quality of the supplementary feeds.

A final flexibility is mentioned with respect to the carry-over of critical feed additives. In case the mill operations and mill design are associated with risks in terms of carry-over of feed material, the problem can be solved with measures in the order of the production of specific animal feeds. If this is not possible, the risk of carry-over should be decreased by having separate pelleting lines and/or the availability of different post-pelleting applications for liquid feed additives. It even may have consequences for the factory design unless different production units are available.

The situations to be flexible in one's own mill design as outlined above for upgrading diet ingredients, end-product forms, supplementary feeds and avoiding carry-over of critical additives urges the need for operating a feed mill in which developments are continuously balanced towards the principles of mill design to meet this flexibility and its effectiveness. This is particularly important for feed mills producing for multiple species of livestock.

10. Technological research based on harmonization; harmonization requirements & research funding

Despite a wealth of data on aspects of feed processing such as particle size and physical pellet quality in e.g. poultry over the last two decades, the published data have been somewhat inconsistent, making it very difficult to draw clear conclusions. Contributing to these discrepancies are various factors such as inherent ingredient variation, specific equipment characteristics, and bird-related factors, although methodological differences employed across laboratories seem also to be a major cause of variation (Mateos et al., 2019). Another major constraint to better understanding the impact of feed processing is incomplete reporting of critical data in some studies. For example, many studies that examined the effect of feed particle size on bird growth performance failed to report feed intake (Reece et al., 1985, 1986; Douglas et al., 1990; Hamilton and Proudfoot, 1995; Kasim and Edwards, 2000; Kilburn and Edwards, 2001, 2004; Charbeneau and Roberson, 2004; Jacobs et al., 2010), which is the primary factor driving growth rate of broilers. This highlights the urgent need for a consensus on the methodology applied to study the different aspects of feed processing and to determine their impact not only on technical issues (feed physical quality and throughput), but also on nutrient utilisation, performance and well-being of the bird.

Since being introduced to the feed industry almost 100 years ago (Coffey et al., 2016), the majority of feed nowadays used globally in the production of meat-type birds is in pelleted form. However, a scan of research published over the past 30 years involving broiler nutrition and management reveals that most results were generated using unprocessed mash diets. Applying these findings to industry, where most of the feed is pelleted (or otherwise processed), is dubious. Early studies can be excused because the true impact of processing on nutrient utilisation was not realised until recently. However, later studies use mash diets simply because many research facilities are not equipped with industry standard pelleting or processing equipment. Indeed, recent evidence indicates that feed form has a substantial impact on amino acid requirements of broilers (Jensen, 2000; Greenwood et al., 2004; Lemme et al., 2006); a scenario that may also apply to energy (Lecznieski et al., 2001) and other nutrients such as calcium and phosphorus. Moreover, almost all available data on the nutritive value of feed ingredients for poultry were determined using mash diets. In addition, feed processing, and more specifically pelleting, affects nutrient digestibility and energy utilisation by birds, and the effect varies depending on feed ingredient and the specific nutrient (Abdollahi et al., 2013, 2014; Naderinejad et al., 2016; Roza et al., 2017; Barua et al., 2019). The

inferences from all the studies above are that values estimated using unprocessed mash diets are not accurate to determine nutrient requirements of birds, and nutritional values of individual feed ingredients in a complete diet fed in pellet form. In other words, extrapolation of some, if not all, findings in poultry nutrition and production research obtained with unprocessed mash diets to an industry situation where most of the feed is pelleted, is questionable. It is, therefore, suggested that determination of nutrient and energy requirements of birds and *in vivo* feed ingredient evaluation in the future should consider the impact of feed processing, and that these data should be generated using pelleted diets to resemble the feeding practice in industry, not only for poultry, but all animal species where feed technology is used in the production system. Moreover, limited published data are available on effects of different processing techniques on various aspects of animal nutrition, and there is an urgent need to better understand their interactions between various feed ingredients in different species. Unfortunately, many universities and research institutions lack on-site feed processing equipment, a factor which severely hampers animal researchers from considering effects of feed processing techniques in their research. Feed machinery manufacturers can play a crucial role by designing and manufacturing small scale feed processing equipment for universities and research institutes, suitable for the small runs needed for research trials. This equipment must resemble the feed processing equipment commonly used in animal feed industry.

A major constraint to conducting research in feed processing is the difficulty to acquire attention and funding for this type of research. A very high proportion of poultry and pig nutrition research today is focused on certain products, mostly feed additives, such as enzymes, pre-/probiotics, phytogenics, essential oils, etc. Whilst this research is vital to the industry, especially in the antibiotic-free production era, and has offered tremendous benefits to the production, health and welfare of production animals and the environment, this focus has obviously impeded other fields of research, such as the effect of feed processing. Considering that most of these feed additives are used in processed feed, mainly in pellet form, research on feed processing, its effect on feed components, and the possible interactions with different feed additives, should be given more attention.

11. Better communication between technologist and nutritionist

Sustainable feed manufacturing is a prerequisite for sustainable farming of healthy livestock. Combining various feed ingredients to supply a specific nutrient mixture to animals brings challenges related to production capacities and feed product quality. Substituting one ingredient with another may be economically beneficial for a feed manufacturer but may influence the nutritional quality and affect production parameters during feed manufacturing. Clear and timely communication between the nutritionist and the feed technologist is essential to optimise nutritional performance of manufactured feeds and the feed production process.

In manufacturing complete feeds, pre-mixtures and feed supplements, various experts work conjointly to meet the end-specifications of the final products. Among others, nutritionists, feed technologists and mill operators are pivotal players in the process. The relationship between the nutritionist and the feed mill operator is of the utmost importance, and their communication is essential to ensure that appropriate ingredients are selected and correctly processed to meet the animal's nutritional requirements and/or physical quality of the feed to meet the specification of the farmer. Mill operators are responsible for monitoring the different processes through state-of-the-art computerized systems, including the review and verification of the end-feed to formulation specifications dictated by the nutritionist. It is good to remember that the operator with their job description have a position that is very close to the clients of the feed mill. Thus, a more complete understanding from both sides related to nutritive values of substitute ingredients and their physical properties are essential.

Feed mill operators and team managers together are responsible for the management of the feed mill and all its processing steps, from ingredient storage to delivery of the final feeds. For the mill operators it is essential to have insight in the role of the nutritionist in the organization. In the livestock industry, performance objectives and feeding practices may be very different between farmers. However, these differences may dictate the nutritional or physical feed quality that is derived from formulation or processability of the diets.

Understanding the manufacturing relationship and nutritional objectives, including compliance specifications, is of paramount importance for a consistent end-product quality. Managers can provide on-site training to ensure a high-quality animal feed and a safe working environment. Vice versa, there is no objection for the nutritionist in reviewing the formulation and product requirements from a technological point of view: also, the nutritionist may require basic and some advanced knowledge of feed processing and its related equipment.

In modern industrial feed mill settings, operators undergo a computer training as well as a safety training to learn how to use computers, implement Good Manufacturing Practices and apply Hazard Analysis and Critical Control Points, as well as implement safety protocols. Additionally, having targeted and tailor-made training courses on nutritional issues regarding different raw materials used for substitution, will provide a meaningful exchange of knowledge and allow discussions with colleagues. Also, understanding the viscoelastic properties of these substituting ingredients will provide nutritionists with knowledge on how to optimize feed formulations in the most competitive and sustainable manner. And as mentioned above, in the future 'big data' will be associated with an approach using machine learning. Senior mill operators can share their vast amount of general knowledge and experience and provide feedback on every stage of the production process. Such quality dialogue between the nutritionists, feed technologists and mill operators may result in a more constant quality of the final feed products together with the lowest electrical and thermal energy consumption during manufacture despite the inclusion of alternative/substitute ingredients.

12. The Internet of Things (IoT) - the gap between science and practice in feed manufacturing

The acceptance and use of smart technologies in society today is widespread. However, the update in the feed manufacturing

industry is sporadic and currently lags behind the advanced manufacturing technologies applied in the computing, automotive and pharmaceutical industries. The world is on the verge of the 4th industrial revolution where increased automated communication between machines through the IoT will drive productivity in the global manufacturing sector. This revolution is expected to impact all disciplines, industries and economies (Marr, 2018). Achieving this transition into the next industrial revolution requires the combination of training for the future feed technologist, and gaining expert knowledge of automated and intelligent manufacturing systems, data analytics, cyber-physical systems and big data. The present level of feed plant automation is moving towards these new challenges.

'Smart manufacturing' and 'Industry 4.0' are the terms most commonly used to describe the next revolution in the manufacturing sector, the former is used in North America and Asia, and the latter in Europe. The key aspects of each are similar: using the six smart manufacturing pillars as defined by Kusiak (2018): 1. manufacturing technology and process, 2. materials, 3. data, 4. predictive engineering, 5. sustainability and 6. resource sharing and networking. The animal feed industry has a variable acceptance and implementation of technology in these pillars with global livestock sector and individual business differences. There are no published data on the level of adoption of integrated technology in the feed industry at a national or global level, although data would prove highly informative to ascertain the state of the animal feed industry in relation to 'Smart manufacturing' or 'Industry 4.0'. Applying these pillars to the feed industry can improve: 1. energy efficiency of feed production, 2. data, environmental and waste management issues and 3. sustainability, with perhaps the most important opportunity coming from shared ownership of production facilities, resource sharing and networking. In a shared environment, while at some level the digital space may be highly transparent, the physical manufacturing assets with their know-how will be protected. This digital-physical separation will allow for shared use of resources across businesses, including the ones that compete (Kusiak, 2018). The traditional models of larger and more efficient production facilities will be challenged, as precision nutrition drives the industry to become more focused on animal requirements at any given production stage. Flexibility will be key as the exclusive feature of "Industry 4.0" is to fulfil customer requirements with product variants in a very small lot size, down to one-off items (Thoben et al., 2017). The integration of feed quality testing systems to the process control system, although already underway, will accelerate as the technology becomes available, affordable and increasingly user-friendly. A product more consistent in physical and nutritional quality shall be the result of this transition.

Leaders with the right balance of skills will be essential to manage organizations through this transition. Employees need to embrace change and realize that their positions and work might be significantly different in the not too distant future. Our education and training systems need to adapt to better prepare people for the flexibility and critical thinking skills they will need in the future workplace (Marr, 2018). The traditional approach to operator training in the feed industry has relied on the use of work instructions, standard operating procedures, and to varying extents the buddy system to develop the operators in the industry. Specific machine operating training, provided by the manufacturer, has been and will continue to be the staple training programs for plant management to use. Although this can be sufficient, when a more detailed insight into the whole process or the industry is required, a different approach will be required. There are several internationally recognized institutes that offer a broader operator, supervisor and manager training to the feed industry with a specific focus on the production process. The future feed technologist shall be expected to achieve a recognized qualification, possibly to diploma level and beyond. These courses must also evolve to provide more detailed teaching on smart manufacturing, integrated process control systems and the future of feed processing. The success of participation in the next industrial revolution from a feed industry perspective will be determined on how well staff are prepared and trained for this evolution.

13. Conclusions

The workshop of the 1st International Feed Technology Conference (IFTC) focused on future directions in feed technology and firstly concluded that animal feed has a social responsibility to contribute to more sustainable food production systems. To achieve this, accurate and fast testing technologies should account for the variability within ingredients and the different practices in feed mills. Moreover, specific aspects of feed manufacturing should be modelled for which big data will play a pivotal role. For future process optimisation, collaboration between skilled data scientists, machine experts, technologists and nutritionists is necessary: novel processing techniques may significantly contribute to improve the nutritional value of raw materials and compound feeds and it is important that structures and functional properties of feed components and their changes with processing are fully understood. Future feed technologists will require recognized qualifications, possibly to a diploma level.

Declaration of Competing Interest

The authors reported no declarations of interest.

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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 (σ) 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].

$$\sigma = \frac{F}{\pi r L} \quad (1)$$

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.

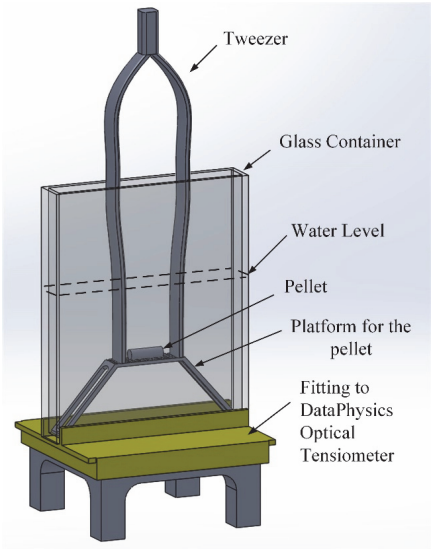

Figure 1. Experimental arrangement for monitoring the swelling of a pellet under stagnant water. The tweezer shown in the figure is used to place the pellet and it is removed during the tests.

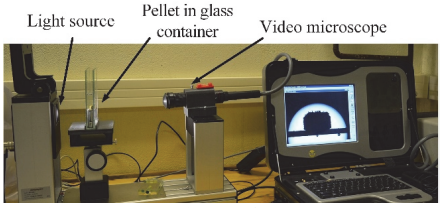

Figure 2. Experimental arrangement to monitor the swelling of pellets assembled in an optical tensiometer

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 (θ) of a single sessile water drop placed on the upper plane surface of a pellet (see Fig. 3). If the initial θ is less than 90°, the surface can be considered as hydrophilic; while θ greater than 90° indicates hydrophobic surface[1, 14].

Contact angle measurements were conducted at room temperature with a video based optical θ 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 θ 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).

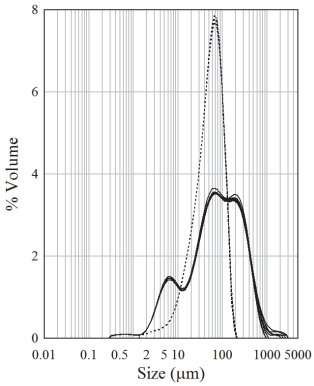

Figure 4. Particle size distribution of microalgae (full line) and lignosulfonate (dashed line).

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.

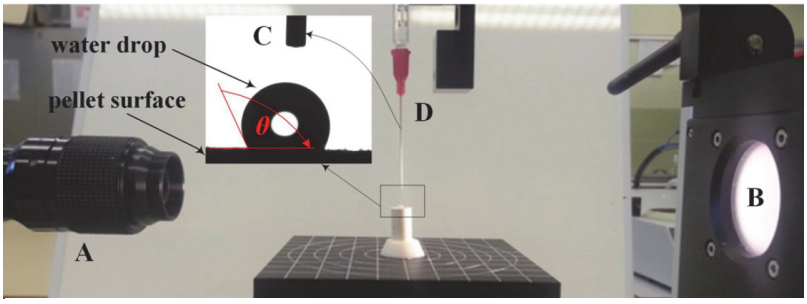

Figure 1. Experimental setup for the contact angle measurements (θ). Letters indicate items as follows: A- camera, B- light source, C- image of a drop on top of a pellet surface for θ tests, D- dosing syringe with a needle[1]

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].

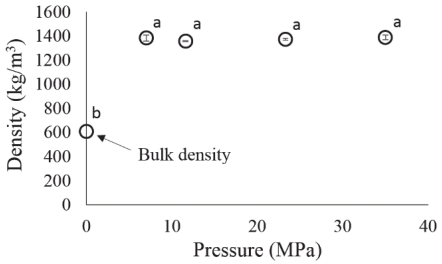

Figure 5. Compressibility of microalgae at different pelleting pressures. Error bars represent the standard deviation. Similar letters indicate no significant differences ($p<0.05$). Averages were calculated from three samples.

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.

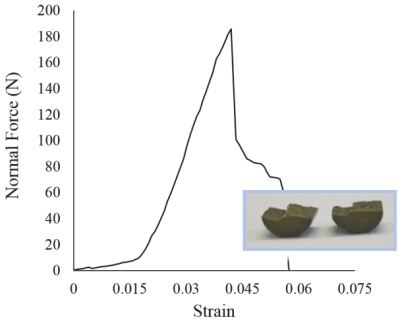

Figure 6. Example of a typical stress-strain curve obtained for the tested specimens. The figure shows at the right bottom a photo of a broken microalgae pellet due to tensile stresses.

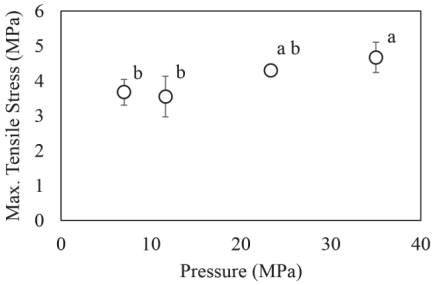

Figure 7. Average tensile strength for pure microalgae at different compacting pressures. Different letters indicate significant differences ($p<0.05$). Averages were obtained from three samples.

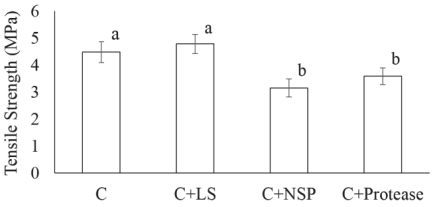

Figure 8. Average tensile strength for the control (C) and when mixed with additives (LS, NSP and protease). Standard deviation is represented by error bars. Averages were obtained from three samples.

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.

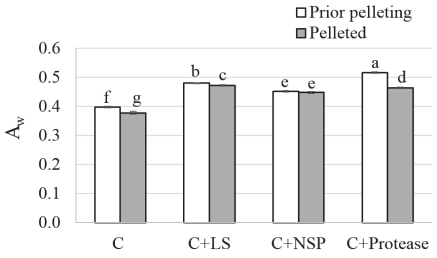

Figure 9. Average water activity (A_w) prior and post pelleting for pure microalgae (C) and when blended with LS, NSP and Protease. Different letters indicate significant differences ($p<0.05$). Standard deviation is represented by error bars. Averages were obtained from three samples

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.

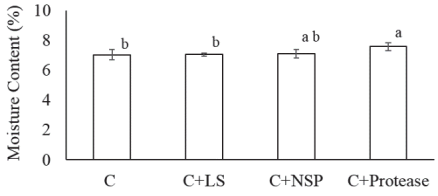

Figure 10. Average moisture content of the mixtures, prior pelleting. Averages were calculated from three samples. Different letters indicate significant differences ($p<0.05$). Averages were obtained from three samples

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.

Swelling of pellets under stagnant water

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.

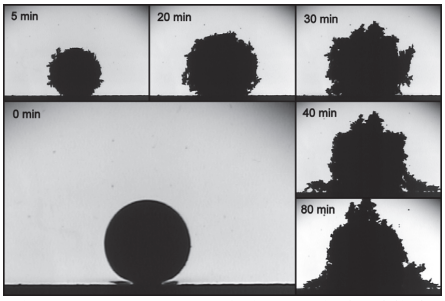

Figure 11. Sequence of pictures in backlight of a pellet under stagnant water from 0 to 80 minutes

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

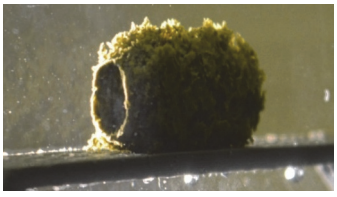

Figure 12. Swollen pellet under water

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.

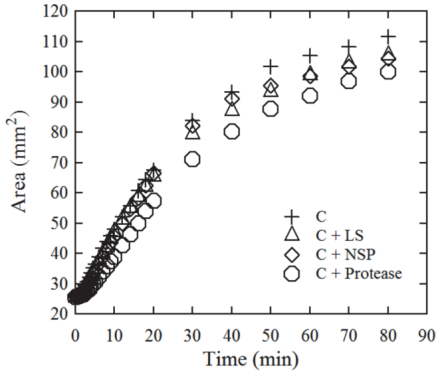

Figure 13. Average cross-sectional area of pellets placed underwater along the testing time.

Surface hydrophilicity and hydration properties of pellets

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

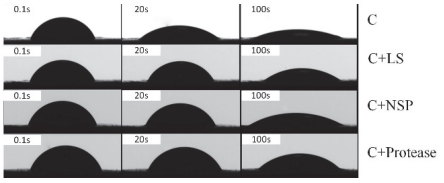

Figure 14. Example of sessile drop at the pellet surface during different time intervals.

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.

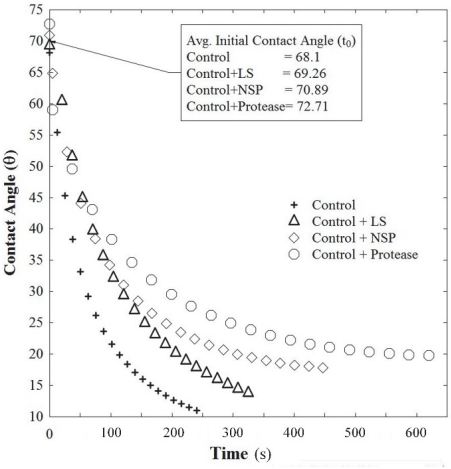

Figure 15. Average contact angles of a sessile water drop placed at the surface of a pellet. Contact angles were measured until total absorption of the water drop.

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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Research article

The effect of feed enzymes phytase, protease and xylanase on pelleting of microalgal biomass

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HIGHLIGHTS

- Enzymes help reducing consumption of the electrical energy during pelleting of microalgae.
- Reduction of flow resistance can be observed when using enzymes phytase and xylanase.
- Hydrolytic activities of the enzymes do not affect hardness of the microalgal pellets.
- Enzymes and their combination improved pellet stability under water.
- Enzymes decrease contact angle degree between pellet surface and oil droplet.

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Feed
Viscosity
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ABSTRACT

Lower energy consumption for producing feed pellets is an important part of the economy in the feed mill. The same is if physical pellet quality is degraded. The interest in using of novel ingredients is increasing due to requirements for the sustainable development goals. Defatted microalgae as by-product from biodiesel production is one of many novel ingredients. The purpose of this experiment was to understand how the addition of small amount of enzymes can reduce the flow resistance in the die during pellet discharge, without affecting the physical quality of pellets. Thus, possibly reduce the total consumption of electrical energy during compaction. Three enzymes, phytase, protease, xylanase, and combinations of those were added to defatted *Desmodesmus subspicatus* microalgae at 3 inclusion levels. Feed enzymes xylanase and phytase helped lowering the flow resistance of the material in the die. Reduction of flow resistance was in average 17 times lover when all three levels of enzyme phytase were used. The same was observed when 0.01% xylanase was added. All feed enzymes and their combination have evidently lowered underwater pellet swelling due to their hydrolytic activity at the surface of the microalgal particles. The hydrolytic activities of the feed enzymes did not affect hardness of the microalgal pellets. Contact angle degree between pellet surface and oil droplet was lowered when xylanase and protease was used at all three dosage levels. However, contact angle degree between pellet surface and water droplets was unaffected by the hydrolytic activity of enzymes.

1. Introduction

Microalgae are an abundant source of protein, carbohydrates, lipids, and antioxidants (Pulz and Gross, 2004). Microalgae have high nutritional value when used for monogastric animal feed (Patil et al., 2005). Use of microalgae may, however, be hampered by components limiting their nutritional value (Bleakley and Hayes, 2017). Phosphate in the microalgal cells has low availability due to binding abilities of phytic acids to bind to cationic elements and protein (Konietzny and Greiner, 2003). Soluble non-starch polysaccharides increase the viscosity of digesta and limits digestion and absorption of lipids and lipid soluble materials (Sinha et al., 2011). These challenges may be addressed by using feed enzymes as digestibility enhancers. Feed enzymes are known to speed up hydrolytic processes during hydrothermal ingredient and feed processing, like steam conditioning of biological materials Such processing can thereby increase the nutritional value of the feed

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ingredients (Hardy, 2000; Svihus, 2010). The use of protease offers promising environmental benefits by enabling improved nitrogen utilization (Oxenboll et al., 2011). Protease showed to change physio-chemical properties of the feed product by affecting the plant-based proteins during and after feed manufacturing. Such change may possibly decrease the energy cost during feed manufacturing (Storebakken et al., 2015).

Reducing use of inorganic phosphorous in feeds, achieved by treating the feed ingredients with phytase (Robinson et al., 2002), can reduce the feed cost. Adding of phytase, combined with protease, has shown to cause improved protein solubility (Bae et al., 2013). Enzymes are used to reduce the energy dilution of the feed by carbohydrates or fibres, derived from vegetable protein concentrates (Hardy, 2000). Enzymes that hydrolyse non-starch polysaccharides (NSP) increase nutritional energy uptake from fibrous materials (Svihus, 2010). According to Miladinovic and Salas-Bringas (2014), the addition of xylanase in fibrous feed mixtures prior to pelleting resulted in a 28 % reduction in the consumption of electrical energy. Reducing die flow reduces compaction of the materials during the pelleting process. All this led to decreased physical quality of the pellets. Function of the enzyme xylanase is to release water from the hydrolysing medium and make it available for protein hydration (Hardt et al., 2014). Thus, when size of non-starch polysaccharides is reduced the general rheological properties of the medium may be changed.

1.1. Enzymatic treatment of microalgae

Microalgal cell walls have a low biodegradability rate (Schneider and Gerber, 2014). In biofuel production, cell wall degradation can be enzymatically increased (Gerken et al., 2013). Enzymatic degradation of the microalgal cell wall could reduce the energy inputs needed for feed pelleting. Influence of the enzymes on swelling of the defatted microalgal pellets, under stagnant water, was previously evidenced by Salas-Bringas et al. (2015).

1.2. Measuring quality characteristics

Animal feeds requires a variety of raw materials mixed in the form of mash prior to being pelleted. Fundamental insights of the processability and physical properties of the single ingredient are necessary to obtain targeted qualities of the pelleted matrix. It is important to evaluate physical properties for every ingredient included in such matrix. The powder-based material compacted into the pellet holds certain microstructures that influence on the texture, durability, hardness (Sun, 2017), hydrophilicity (Güttler et al., 2013), lipophilicity (Cao et al., 2007), and water activity of the final pelleted products (Roca et al., 2006).

Physical characteristics of the materials and their rheological properties can help in determining functionality of the ingredient during product development, shelf life estimation, evaluation of the texture, or overall product quality control (Stokes et al., 2013). Resistance of the mixed material to deformation during pelleting can influence consumption of electricity, final shape, texture, and physical quality of product. Quality parameters are dependent on the time scale of the deformation process, shear, and thermal history during manufacturing (Chen and Engelen, 2012). Controlled processing variables such as shear and temperature can influence the structure of the final products (Hermansson, 2000).

It is important to know how feed ingredients contribute to flow behaviour during compaction, final texture, and physical quality of compacted feed matrixes. Such knowledge sets manufacturers in a position to rationally design better nutritional and physical quality products prior to commercial manufacturing. For instance, the texture of feed products is physically and chemically dependent to various ingredients and additives that are influenced by temperature, dwell time and shear. This defines all mechanical, geometrical, and surface attributes of a final product.

Physical behaviour in the network of the food-polymer is influenced greatly by small particles, under 100 μm (Aguilera, 2005). Microalgae vary in their size. Such size variation may influence physical characteristics and overall texture and viscosity of the feed-products.

Physical quality properties of the dry and solid feed compacts, as hardness and durability, are important parameters for reliable quality measurements (Thomas and van der Poel, 1996). Under mechanical stress, the strength of feed pellets can be evaluated, and thus, the behaviour of the final product during storage or transportation can be assessed. The mechanical properties can be used to evaluate the physical contribution of a single feed material to the entire diet formulation under steam conditioning (Maier and Gardecki, 1993) or manufacturing parameters (Briggs et al., 1999).

Water activity (a_w) is a good indicator of the shelf life of feed products. Most degradation reactions are related to a_w. Proteins, carbohydrates and NSP quickly interact with water and increase the a_w. Such feed elements can lower their water pressure by polar-binding in small molecules or by surface interactions in large molecules (Maltini et al., 2003) when interacting with the enzymes. Therefore, it is crucial to understand how certain feed material react with water to predict their behaviour in the feed. The thermodynamic effect in a raw ingredient could control the texture of the final product at a given a_w during pelleting.

When developing a feed matrix, a major challenge can arise from the incompatibility of ingredients having different surface properties. Surface hydrophilicity and lipophilicity of feed pellets play an important role in post-manufacturing processes. The contact angle (θ) and surface energy of liquids can differ in each compacted medium, hence, the final product quality may also differ. The surface energy of a compacted medium can control the level of physical interaction between particles by adsorption, adhesion, friction, and wettability of the surface (Yuan and Lee, 2013). If the initial $\theta < 90°$, the surface can be considered as hydrophilic, whereas $\theta > 90°$ indicates a hydrophobic surface (Förch et al., 2009; Mišljenović et al., 2015).

Poor mechanical properties of the feed pellets may be expected if the feed ingredients have little or no interactions through chemical and mechanical bonding (Saheb and Jog, 1999). Chemically similar feed ingredients, originating from the same ingredient type, may have different initial water θ characteristics (Roman-Gutierrez et al., 2003; Güttler et al., 2013). The wettability of the ingredients can be evaluated by measuring the θ of any given liquid droplet placed at the surface of the compacted pellets. Nevertheless, the structural differences in compacted solids could alter θ. Different particle sizes in a feed matrix can influence porosity of the pellets (Vukmirovic et al., 2017). The θ measurements depend on liquid diffusion inside particles and liquid absorption in the voids between the particles (Roman-Gutirrez et al., 2003). Initial θ values and the droplet age of water and oil (i.e., at the time when the droplet is placed at the surface) were considered in this experiment to evaluate the contribution of enzymes to surface wettability of compacted microalgae.

The underwater swelling rate (UPS) is a quality parameter to indicate how long a pellet could remain consumable and nutritionally useful by farmed animals underwater. Slow UPS is an important requirement for shrimp feeds, due to the slow eating habits of the animals. Therefore, UPS is a quality indicator of how pellets remain underwater until they are consumed. Also, UPS measures particle detachment within the pellet structure, linked to the swelling rate. It has been a common practice to measure disintegration of pellets underwater. This is measured by several methods, commonly known as “pellet water stability”. Obaldo et al. (2002) reviewed these methods as the horizontal shaking, static water method and vertical shaking. Measuring the swelling rate of the aquatic feed pellets submerged in stagnant water is vital for evaluating the possible leaching of micronutrients from the feed into the water. Leaching of nutrients from the feed, for example nitrogen, could lead to a decreased water quality and thus health risks for the farmed animals. Newly developed methods for measuring swelling rate of the feed pellets through image analysis may give more precise answer to disintegration of the pellets under water (Salas-Bringas et al., 2015).

The need for mapping the flow properties during pelleting, physical quality of pelleted products when including novel feed materials, and the effects of enzymatic additives, can be used to indicate the economic benefits of utilizing the enzymes. Such tool may help to decision makers where the emphasis is set towards sustainability, without threatening the final feed quality outputs. Usage of enzymes during feed manufacturing demonstrates a large potential for better use of feed constituents and their function (Bedford and Partridge, 2003). There is no published data on how the addition of enzymes correlates to the physical quality of compacted microalgal biomass. There is no reported evidence of how the enzymes during preparation of the microalgal biomass, as a feed material would influence production capacities and electricity consumption during manufacturing. The overall aim of this work was to understand the flow of the novel material when a single enzyme or a combination of enzymes, in various dosages and at low water content is added to microalgal biomass. If in low water-content environment the enzymes are altering chemical properties of compacted defatted microalgal materials, that could all lead to changed flowability of the material during pelleting. If the flowability changes the power consumption changes too by different pressure at incipient flow (p_{max}). This can all possibly lead to altered stability of pellets underwater, hydrophilicity or lipophilicity as well as physical strength of the compacted microalgal material.

Objectives of this research are based on the hypothesis that different enzymes and different dosages can lower the p_{max} of microalgal biomass during pelleting. Such change could allow the particles from microalgal biomass to pack better during compaction and that would further contribute to harder pellets. Harder pellets would maintain longer their structure if submerged underwater and will be longer available to intended usage, as for example feeding shrimps. In connection to better packing of the particles due the enzymatic hydrolyses this all may contribute to lower acceptability of oil or water to penetrate surface of the pellets.

2. Materials and methods

2.1. Characteristics of the experimental materials

Microalgal biomass of *Desmodesmus subspicatus* (Cellana, Kailua-Kona, HI, USA), as residual biomass from microalgae oil processing, was used.

The percentage of enzymatic dosage added to the biomass was based on the total batch size of 200 g dry matter. Each batch was weighed on a microscale (Mettler Toledo, model LJ16). Enzymes supplied by AB Vista (Marlborough, UK) in liquid form were beta 1–4, endo-xylanase (Econase XT), an *E. coli* derived phytase (Quantum Blue) and *Fusarium equiseti* protease (non-commercial product). The enzymes were applied in three different dosages. The recommended dosage (*R*) for all enzymes, was based on the optimal bioactivity of the enzymatic additives, as recommended by the supplier. All presented values are units of weight %. The R for xylanase was 0.01 %, half dosage (*H*) 0.005 %, and double dosage (*D*) 0.02 %. For phytase the dosages were R - 0.03 %, H - 0.015 %, and D - 0.06 %. For protease, R - 0.006 %, H - 0.003 %, and D - 0.012 %. Each dosage of enzymes was mixed with 2.5 % distilled water and thereafter sprayed over the biomass to ensure good mixing.

2.2. Preparation of the experimental mixtures and sampling

An intensive mixing of the biomass was performed to distribute well enzymes by using a high shear mixer, having three impellers and one tulip-form chopper (Diosna P1/6, Osnabrück, Germany). The mixing speed of the impellers was 250 rpm, and the chopper speed was 500 rpm. Spraying of the distilled water/enzyme solution was performed with a spraying lance (Model 970, Düsen-Schlick GmbH, Germany) assembled in the mixer. Samples for moisture analyses were taken from random areas in the mixer for each batch, three samples per batch. Thereafter, all three samples for each trial were mixed together to obtain a representative sample, assuming a homogeneous mixture. The moisture content presented in this work is an average of three measurements. Moisture was measured by the standard method (EU, No. 152/2009) and the average moisture measurement for all trials was 8 % w/w (+/- 0.3 %). Immediately after mixing that lasted for 60 s the water/enzyme solution was added and thereafter all the powder was collected, and vacuum packed to prevent moisture loss. The samples were stored at 4 °C for a maximum of 4 weeks until pelleting.

2.3. Pelleting

A single pellet press method (Salas-Bringas et al., 2010, 2011; Salas-Bringas, 2011) was used for compacting approximately 0.2 g of the microalgal biomass into cylindrical pellets (Figure 1).

The enzyme treated microalgal powder sample was poured into preheated die comprising channel. The temperature in the die was 81 °C as recommended to eliminate possible salmonella contamination (VKM, 2006). The material poured into the die was preheated for 3 min prior to compaction. Compaction was done with a compression rod with a diameter of 5.45 mm. The compression rod was set in the die immediately after the powder was poured, to prevent release of water from the sample during heating. When the sample reached a desired temperature, an initial pre-load pressure of 240 kPa was applied to the sample in the pelleting die before compression of the material started. This was done to rearrange particles of the microalgal biomass and avoid creating the air pockets in the die hole, without the risk of compaction. Pelleting was done by applying maximal force load of 285 N. Calculated compressibility of approximately 12 MPa was applied to microalgal biomass. The chosen compacting pressure of the trial mixture was used according to densities of products derived from the commercial animal feed ring-die pelleting process Salas-Bringas et al. (2011). The compaction speed was set to 10 mm/min through a rod inserted in a 5.5 mm compressing channel of the blank die. After compaction, the blank part of the die (i.e., closed end) was removed and thereafter, the pellets were discharged. Discharging of the pellet was done with a speed of 2 mm/min, which was low enough to avoid exceeding the compacting-pressure and hence avoid any further compaction. Total retention time of the materials in the compressing channel of the blank die was 9 min. The compacted pellets were 5.5 mm in diameter and a weight of 0.2 g. All compacted pellets were stored at 4 °C for a maximum of 30 days prior to analysing of physical properties.

2.4. Analytical techniques and measurements

2.4.1. Particle size distribution

A Malvern Mastersizer S instrument (Malvern Instruments Ltd, Worcestershire, UK) utilizing a laser diffraction method was used to determine the particle size distribution of the microalgal biomass. Combination of wet-sieving and laser diffraction method was used to determine final size of the particles. Untreated microalgal sample and its replica was placed in a particle dispersion unit one after another. The cell where the microalgal sample was located in the path of the beam of the scattered light. The sample was circulated through the cell that had depth of 2.4 mm. The setting of the focal length in the detector was 300 mm. The detector consisted of 44 photosensitive rings. Fraunhofer diffraction theory for spherical particles was used to calculate volumetric particle size distribution of the light energy on the detector (Allen, 1997).

The particle size distribution analyses of non-treated samples showed a particle size in the range of 1.45–549.5 μm. About 10 % of all particles had average size lower than 14.6 μm, whereas 50 % of particles were lower than 83.3 μm and 90 % of particles were under 275.5 μm. A De-Brouckere mean diameter volume of the particles for the representative sample had a diameter of 32.3 μm and specific surface area 0.19 m^2/g.

2.4.2. Compaction and pellet discharge

The observations during compaction and pellet discharge from the die were measured as maximal compaction pressure (Pa) as a product of

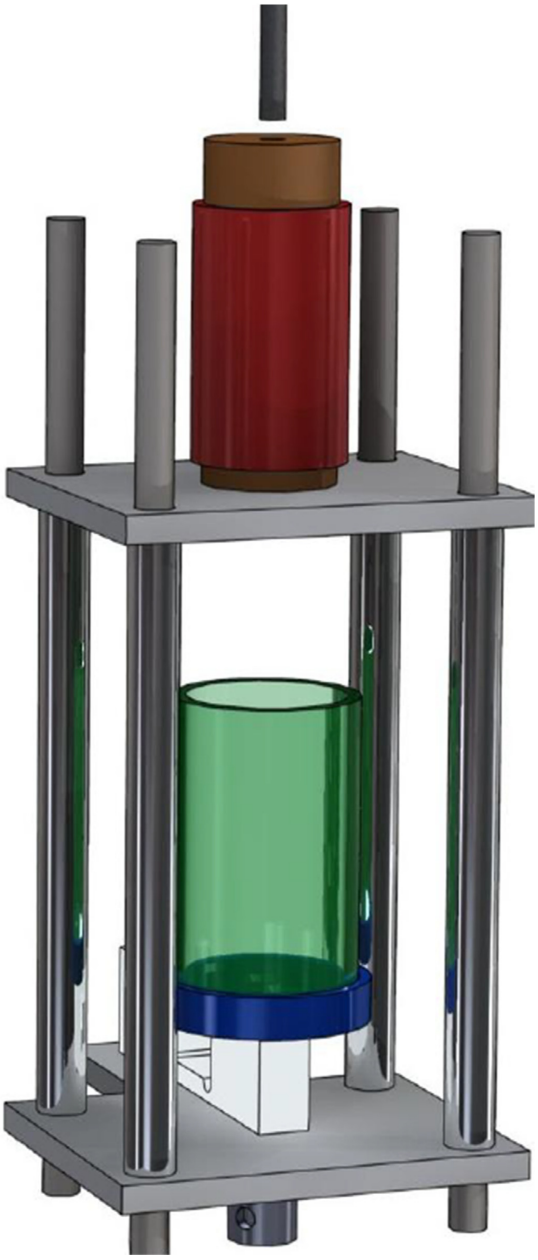

Figure 1. Single die pellet press. Adopted from Salas-Bringas et al. (2011).

maximal compacting force (N) divided by the area of a compact (mm^2). The results were recorded with NEXIGEN Plus software, attached to a Lloyd texture analyser (LR 5K Plus; Lloyd Instruments, U.K.).

2.4.3. Pressure at initial flow measurement

The pressure required to initiate pellet discharge from the die once the blank die was removed is referred to as the pressure at incipient flow (p_{max}). The p_{max} was recorded to quantify differences generated by friction on the die-pellet contact area. The die flow of the compressed pellets was analysed by recording the maximal pressure needed for the pellet to start flowing through the open die-diameter. Measurements were performed immediately after compaction and when the blank die was removed. The set discharge speed (2 mm/min) at the pelleting rig was set to ensure that the releasing pressure would not reach the compaction pressures. Such measurements indicate possible changes in electrical energy consumption due to changes in the resistance of the material to flow through the die. The analytical results for the tensile stress and maximal force prior to when the pellet started flowing through the die were further estimated with Eq. (1).

$$p_{max} = F/\pi r^2 \quad (1)$$

where F is the load needed for the pellet to start flowing and r is the radius of the pellet and π the constant ratio of the circumference of the circle to its diameter.

2.4.4. Tensile strength

Tensile strength analyses were obtained through maximum force (F) applied on cylindrical specimens under diametral compression, on three randomly chosen pellets for each treatment. Strength for each pellet was measured by the first peak F occurring during a diametral compression at speed of 1 mm min^{-1}. For brittle cylindrical specimens, the stress (σ) was estimated by using Eq. (2) better known as the Brazilian test. The tensile fracture was produced in a pellet by compressive loading across the diameter. The σ measurements were done by using a probe with a flat surface of 60 mm in diameter, connected to a texture analyser (Lloyd LR 5K Plus, Lloyd Instruments, U.K.).

$$\sigma t = (2F/(PiDl)) \quad (2)$$

where σt is the maximum tensile strength (MPa), F the load at fracture (N), Pi is constant ratio of the circumference of the circle to its diameter, D is pellet diameter (mm), and l is measured pellet length (mm).

2.4.5. Water activity (a_w) measurements

The change of a_w was determined by use of a HygroLab™ C-1 instrument (Rotronic AG, 8303 Bassersdorf). Three randomly chosen pellets for each treatment were used. The average temperature during a_w measurements was 23.5 °C (±0.4), and relative humidity (RH) of the air in the measuring cell was 37 % (±2) for all samples. The procedure for a_w measurement is explained in the user manual (IN-E-HyLab-V4_11) for Rotronic a_w devices (Rotronic AG, 8303 Bassersdorf).

2.4.6. Contact surface angle (θ) measurements

Characterization of the surface wetting of the pelletes with oil and water and was measured with an optical device OCA 15EC (Dataphysics Instruments GmbH, Germany). The θ measurements were made on three randomly chosen pellets, for each treatment, to compare how wettability of the pellets could be influenced by enzymes. The initial θ is defined as the moment when the drop is placed on the surface of the pellet (T0). The θ measurement was made with distilled water and rapeseed oil. The droplet volume for distilled water and rapeseed oil was 1 μl and 5 μl, respectively. To detach the oil droplet from the needle, a larger volume was needed, due to the larger surface tension of oil (Esteban et al., 2012). The droplets were disposed from a dosing system on the upper plain surface of a pellet (Figure 2). The recorded droplet absorption is presented in the results as the initial θ (T0) and final droplet-age, measured in seconds. Water and oil θ were recorded within 1.2 and 3 s, respectively. After those periods, the droplets fully penetrated the pellet. The measurements were carried out at a temperature of 20 °C (±2). The change of θ was observed by SCA20 software (Dataphysics Instruments GmbH, Filderstadt, Germany).

2.4.7. Underwater pellet swelling rate (UPS)

To monitor the swelling rate of a pellet under stagnant distilled water, a special arrangement was designed (Figure 3). The UPS measurements were made by assembling video microscope (microviper) with a

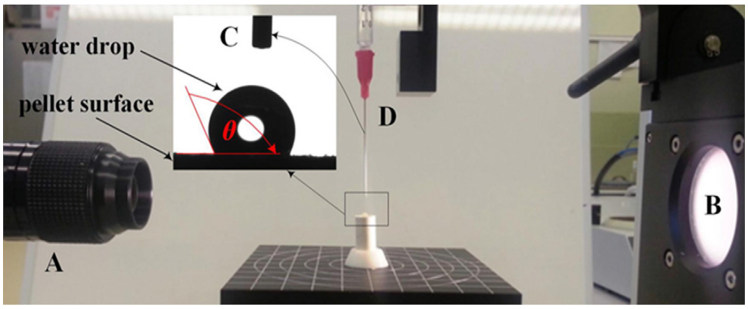

Figure 2. Contact surface angle measurement setup. Different letters indicate: A - video camera; B – light source; C – image of a drop on top of a pellet surface; D – dosing syringe. Adopted from Mišljenović et al. (2015).

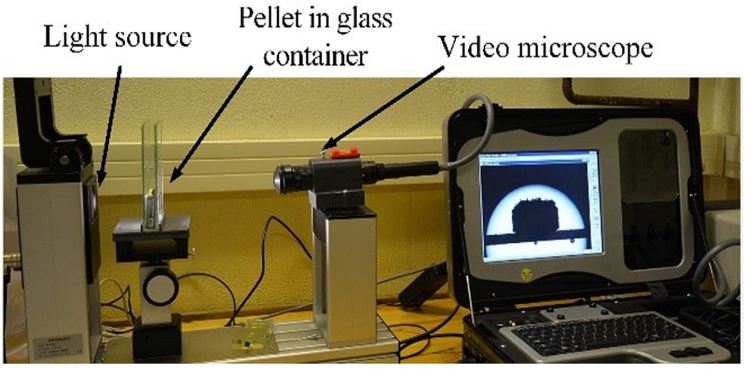

Figure 3. UPS experimental setup using image analysis (Salas-Bringas et al., 2015).

microscope lens (Allen ¼") into the optical tensiometer (OCA 15EC, Data Physics Instruments GmbH, Filderstadt, Germany) as shown in Figure 2. The image processing software Fiji (open source under the GNU, General Public License) was used for the optical monitoring of pellet swelling, according to Ferreira and Rasband (2012). Distilled water at a temperature of 19.5 °C (+/- 1) was added to the experimental glass container. Thereafter, a randomly chosen pellet was used for each treatment. The measurements were made in triplicate. A cross-sectional view of the non-swollen pellet with a diameter of 5.5 mm was used as a starting point. A picture was taken every minute for a total observation time of 14 min. The duration of the observation time was chosen as the time required for shrimp to find the pellet in open pond shrimp farming (Lovell, 1998). The total observation area occupied by the cross-section of a recorded pellet in the water during a total observation time of 14 min represents the UPS results.

2.4.8. Measuring of soluble protein and phosphorous

Phosphorous content was measured with a spectrophotometric method (FAO, 2011) with simplified sample preparation consisting of grinding the pellets with pestle and mortar. Soluble protein measurement was done on samples having lowest flow resistance of the pellets in the die (p_{max}). Soluble protein and phosphorous measurements were not performed on other samples because there was no observed change of the p_{max} in those samples. Compacted material treated with phytase was compared to control samples. Samples were held overnight on 550 °C temperature. Ash samples were cooked in the low acidic solution for total release of phosphorus and thereafter diluted in water. Samples are added to various reagents to obtain a colour reaction which was read spectrophotometrically. Measuring of soluble protein was done based on Licitera et al. (1996). During protein extraction, a stable pH and temperature was secured. A borate phosphate buffer with pH 6.75 was used to hold samples at 39 °C during incubations.

2.5. Data analyses

Variables were analysed as a 5 (enzyme treatments) x 3 (enzyme dosages) factorial arrangement in a randomized complete block design. Software used for plotting was Microsoft Excel and Minitab v.17 for statistical analyses. One-way analyses of variance (ANOVA) was used to examine possible effects of the enzymes and their dosages on the responses. Significant differences between treatments were determined by the Tukey–Kramer method, using a 95 % confidence interval. To analyse the existence of correlations between variables, a Pearson correlation test with a 95 % confidence interval was used.

3. Results

3.1. Flow resistance in the die during pellet discharge

Measurement of p_{max} showed that various enzymes and their combination influenced changing the resistance of the material to the flow in the die ($p < 0.05$). The lowest and significantly different p_{max} was observed for phytase and xylanase. ($p < 0.001$) (Figure 4).

All other enzymes, their combination and dosage level did not show to influence p_{max}.

3.2. Strength of pellets

The tensile strength of pellets was not influenced by adding the different enzymes independent of dosage either when added combined

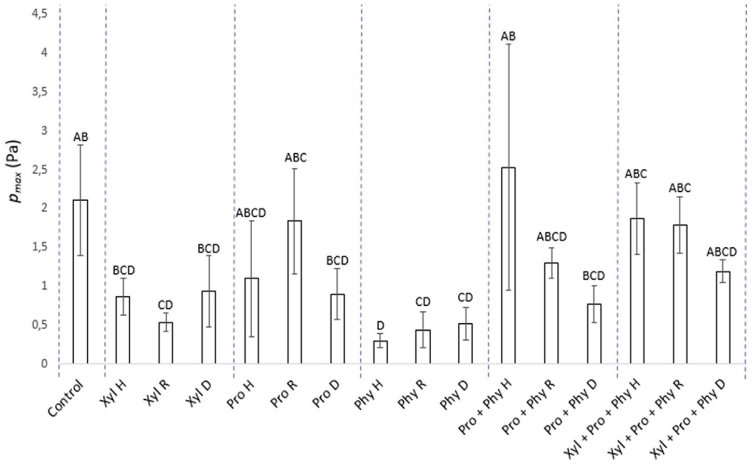

Figure 4. Mean values for p_{max}, derived from 3 replicates. H, R and D indicate half; recommended and double dosage, respectively. Xyl, Pro and Phy are enzymes xylanase, protease and phytase, respectively. Means that do not share a letter are significantly different, according to the Tukey pairwise comparisons test. Letters are organized in descending order and show significant differences ($p < 0.05$). The error bars represent 95 % confidence intervals.

enzymes or single enzymes (Table 1). No relationship between pellet strength and p_{max} was observed ($p = 0.82$; $r^2 = 0.011$).

3.3. Surface contact angle (θ) measurements for water and oil

Type and dosage of added enzymes did not have any influence on the initial θ (T0) either of water nor oil (Table 1). Enzyme type and dosage did not influence T0 oil θ. However, by having the oil droplet age for three seconds, xylanase and protease showed increased lipophilicity of the compacted microalgal biomass, when compared to the control treatment and treatments with different dosages of mixed protease and phytase (Table 1). Phytase alone or in combination with other enzymes, independent of the dosage, did not influence θ, when compared to the control treatment at after 1.2 s for the water or 3 s for the oil. The same was observed when mixture of protease and phytase was used or when all enzymes mixed together were added in various dosages. Increasing the dosage of all enzymes mixed together did not have any influence on oil θ at 3 s.

3.4. Underwater pellet swelling rate (UPS)

When comparing enzymatic treatments with the control treatment, it can be concluded that pellets treated with single enzymes or mixture of enzymes had decreased UPS (Figure 5). The total observation circular area of a non-swollen pellet was 23.7 mm^2. The slowest UPS 24.6 mm^2, for one minute was observed in treatment with phytase (Figure 5C). However, this was significantly different ($p < 0.001$) only by comparing the control treatment and the double dosage of mixed protease and phytase. Control treatment had UPS of 35.5 mm^2 and mixed protease and phytase 34.9 mm^2 (Figure 5D). Lowest UPS was observed at 14 min for a treatment with xylanase/protease/phytase added as a double dosage (Figure 5E). The highest UPS was observed in the treatment with

Table 1. Mean values and standard deviations for contact angle analyses (water and oil), pellet tensile strength and water activity (a_w).

Treatments	Dose level	CA oil; T_0	CA oil; 3 s	CA water; T_0	CA water; 1.2 s	Tensile strength (MPa)	a_w
Control	-	44.8 ± 13.3	34.7 ± 5.4 a	52.0 ± 16.2	29.6 ± 17.6	2.4 ± 0.7	0.3 ± 0.01 b,c
Xylanase	H	56.1 ± 4.3	21.4 ± 1.8 c	48.7 ± 5.5	20.6 ± 3.8	1.6 ± 0.2	0.2 ± 0.01 d
	R	51.6 ± 3.2	20.4 ± 3.1 c	58.1 ± 7.4	23.6 ± 4.4	1.3 ± 0.5	0.2 ± 0.01 d
	D	53.1 ± 14.2	21.8 ± 3.7 b,c	49.1 ± 8.8	17.5 ± 4.0	1.6 ± 0.3	0.3 ± 0.01 d
Protease	H	51.9 ± 6.0	19.1 ± 2.6 c	47.6 ± 2.6	26.0 ± 14.0	1.5 ± 0.3	0.2 ± 0.01 d
	R	53.9 ± 9.9	22.4 ± 1.2 b,c	43.6 ± 2.2	28.3 ± 6.1	2.0 ± 0.4	0.3 ± 0.01 d
	D	45.5 ± 2.4	22.1 ± 0.8 b,c	48.8 ± 0.4	29.2 ± 0.7	2.0 ± 0.4	0.2 ± 0.01 d
Phytase	H	46.6 ± 4.3	29 ± 4.7 a,b,c	44.8 ± 4.8	17.3 ± 2.4	2.7 ± 0.7	0.2 ± 0.01 d
	R	57.2 ± 6.7	27.7 ± 5.1 a,b,c	46.3 ± 2.2	15.6 ± 1.6	2.0 ± 0.3	0.2 ± 0.01 d
	D	52.2 ± 6.9	29.2 ± 4.9 a,b,c	48.4 ± 13.3	15.8 ± 2.5	2.4 ± 1.3	0.3 ± 0.02 c,d
Protease/Phytase	H	54.2 ± 3.8	39.4 ± 12.6 a	41.7 ± 3.4	16.1 ± 3.3	2.7 ± 0.3	0.4 ± 0.04 a
	R	51.1 ± 6.6	38.2 ± 3.9 a	40.0 ± 2.0	16.2 ± 5.0	2.0 ± 0.4	0.4 ± 0.06 a,b
	D	49.3 ± 6.7	34.7 ± 3.8 a	43.1 ± 3.7	17.2 ± 0.9	2.7 ± 0.7	0.4 ± 0.03 a,b
Xylanase/Protease/Phytase	H	47.0 ± 2.1	36.2 ± 0.8 a	51.2 ± 6.6	31.4 ± 6.5	2.2 ± 0.8	0.4 ± 0.04 a,b
	R	48.4 ± 5.2	32.7 ± 5.0 a,b	44.2 ± 6.8	21.5 ± 2.6	2.4 ± 0.9	0.4 ± 0.04 a,b
	D	41.9 ± 8.6	27.1 ± 2.9 a,b,c	57.5 ± 9.9	31.0 ± 3.3	1.9 ± 0.3	0.4 ± 0.06 a,b

Results without letters are not significant ($p \geq 0.05$).
H - half dosage; R – recommended dosage; D - double dosage. Means that do not share a letter are significantly different for the respective analyses, according to the Tukey-Kramer test (0.05). Letters are organized in descending order and show significant differences ($p < 0.05$) ± confidence intervals.

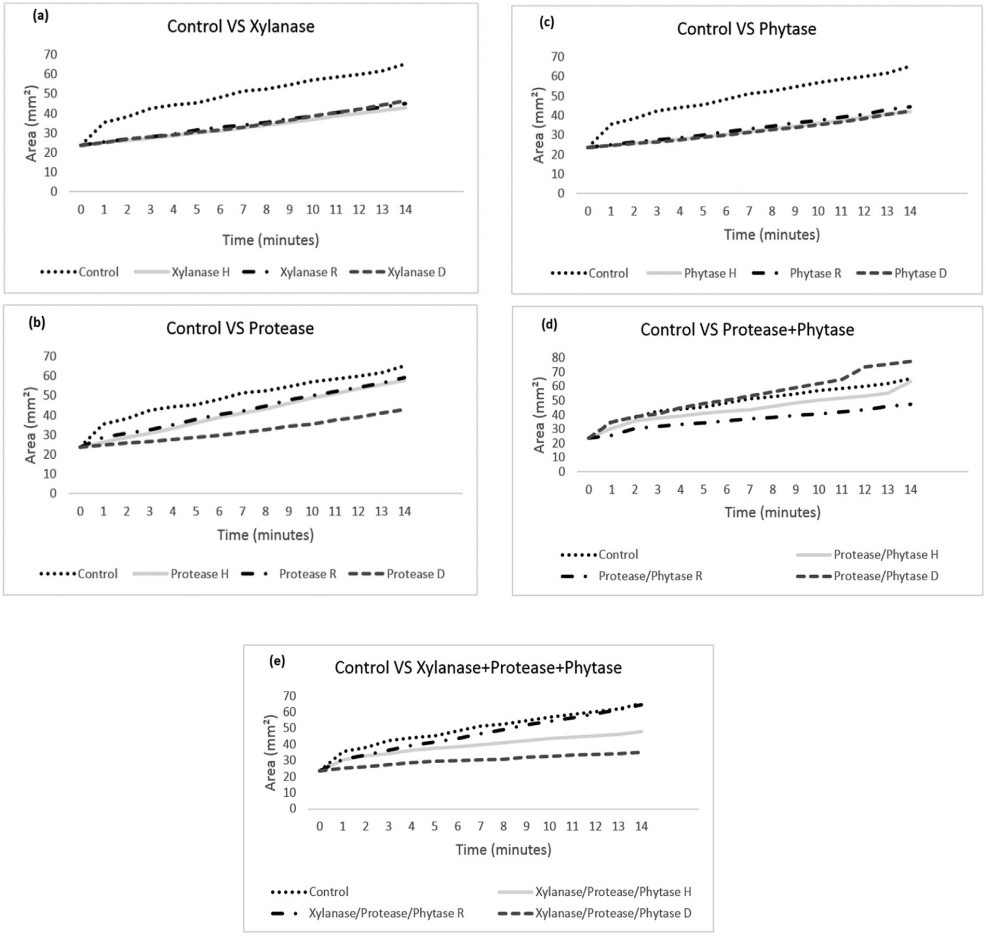

Figure 5. Effect of enzymes and their dosage level on pellet swelling under stagnant water, observed within 14 min, and compared to control. (a) xylanase, (b) protease, (c) phytase, (d) protease and phytase, (e) xylanase, protease and phytase.

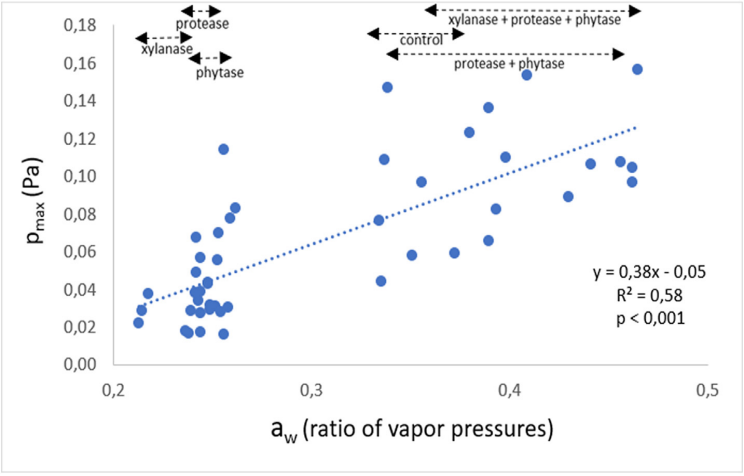

Figure 6. Water activity in direct correlation with compacted biomass flow (p_{max}) through cylindrical die.

protease/phytase (Figure 5D). At the first minute, the highest observed swelling was in the control treatment (Figure 5A) and the lowest was for the phytase H (Figure 5C). Five minutes of submerging a pellet under water, the largest swelling was observed in treatments with a protease/phytase double dosage and the control treatment (Figure 5D). However, by adding the protease/phytase mixture at the recommended dosage, after 6 min of UPS observation, a significant decrease ($p < 0.001$) of pellet swelling was observed (Figure 5D). When compared to the control, the treatments with xylanase were significantly lower ($p < 0.001$) in UPS, despite the dosage level (Figure 5A). The same was observed for treatments with the enzyme phytase (Figure 5C).

3.5. Water activity (a_w)

All enzymes, except the phytase D, added to microalgal biomass independent of dosage levels influenced a_w values when added separately. However, when added as mixes of enzymes no influence of a_w value was observed, except the mix of protease and phytase H (Table 1).

There was a correlation between a_w values and p_{max} ($R^2 = 0.46$) (Figure 6). By increasing a_w, the p_{max} values also increase. Additionally, by having higher a_w values, the tensile strength of the pellets increases ($R^2 = 0{,}12$) (Figure 7). However, the trend for this is unclear.

There was no correlation between a_w values either and to oil θ nor to water θ ($p > 0.05$)., The increased UPS rates were not correlated with changed a_w values ($p > 0.05$).

3.6. Effect of enzymatic hydrolyses on chemical change of pelleted microalgal material

Total phosphorous content did not influence flow resistance of the pellets (Table 2). When comparing to the control sample the soluble protein content was significantly higher in samples treated with phytase. Different dosages of phytase did not influence the changes of total soluble protein measurement in the pellets when compared to control (Table 2).

4. Discussion

The observed reduction of p_{max} for the recommended dosage of xylanase and all dosages of phytase may be explained by changes in the chemical structure of linearly layered polymers in the cell wall, well defined as xylans (Bajpai, 2014). Xylanase may hydrolase the bonds between xylans in the cell walls, decreasing the resistance to motion and friction.

Table 2. Chemical change influenced by the treatments: Total phosphorous and crude protein levels.

Treatments	Dose level	Total P (mg/g)	Crude protein (g/kg)
Control		1.83	55.1 a
Xylanase	H	1.56	-
	R	1.31	-
	D	1.47	-
Protease	H	1.32	-
	R	1.51	-
	D	1.42	-
Phytase	H	1.31	60.9 b
	R	1.41	58.8 b
	D	1.36	59.9 b
Protease/Phytase	H	1.08	-
	R	1.41	-
	D	1.39	-
Xylanase/Protease/Phytase	H	1.84	-
	R	1.43	-
	D	1.39	-

Results without letters are not significant ($p \geq 0.05$).

Also, the reduction of p_{max} is rationalized by hydrolysis caused by phytase. It was expected that phytase will influence structural changes of the phosphorous that is stored in the form of inorganic polyphosphate during "luxury phosphorous uptake" (Solovchenko et al., 2019). Polyphosphates are of pivotal importance in microalgae (Grobbelaar, 2004), and covalent binding to proteins that enable microalgae to acclimate to stress conditions (Sanz-Luque et al., 2020). However, according to the results presented in this research work this was not the case (Table 2). The experimental treatments did not provide any evidence that type of enzyme or its dosage is influencing any change in availability of phosphorus in the pellets (Table 2). Accumulated inorganic polyphosphate in *Desmodesmus sp.*, remain in the microalgal biomass, even after breaking the cell wall (Xin et al., 2010). By introducing phytase, the hydrolyses of the links between polyphosphate and protein occurs. Such hydrolyses reduced viscosity due to increased level of soluble protein, as showed in Table 2. Phytase, whether based on histidine acid or with alkaline base, can catalyse hydrolysis of a wide range of molecules containing phosphorous (Oh et al., 2004). Phytase, thus, enables the detachment of phosphate from a wide range of molecules. During such detachment the

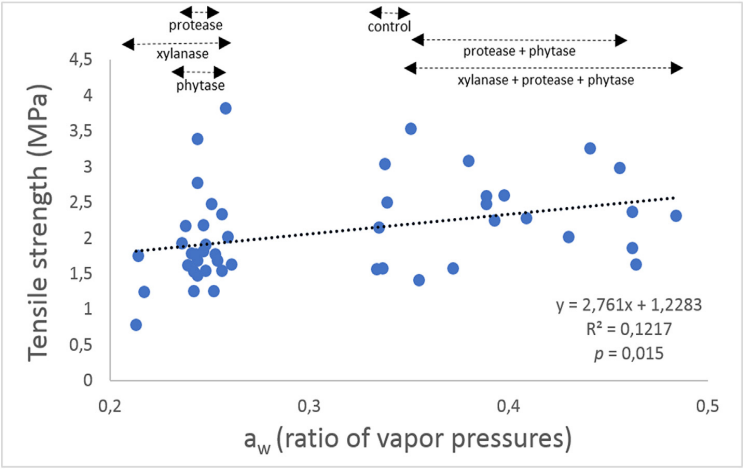

Figure 7. Pearson correlation between water activity and tensile strength.

chemical nature of protein surfaces may change (Hunter, 2012). When phosphate ester bonds detach from the protein, the protein may decrease the viscosity if being compressed by the forces, thereby, lowering p_{max} during pelleting.

Mixing phytase and protease did not significantly decrease p_{max}. This contrasts with Bae et al. (2013). The reduced p_{max} values, obtained when phytase was added to the microalgal biomass and thereafter pelleted, could be ascribed to hydrolyses, and changes of the chemical bonds in lipoprotein-complexes. Doubling the dosage of phytase, protease, combination of phytase and protease and all three enzymes combined did not affect p_{max}. Every enzyme in the combined enzymatic cocktails potentially had some influence on the rheological properties of microalgal biomass. Enzyme xylanase hydrolysed the xylans present in the cell walls, protease influenced protein solubility and phytase influenced phosphate ester bonds. Enzymes xylanase, double dosage and phytase all three dosages partially influence the change of complex molecules in microalgal biomass connected to the cell wall. This was evident as during compaction lowered p_{max} values. Such changes are directly influenced by catalysing the hydrolysis of the bonds between fibre and peptidoglycans from the cell walls (Bae et al., 2013). However, there was no indication that mixtures of xylanase, protease and phytase, in different dosages, influenced p_{max} when compared to control. One probable reason for the unchanged p_{max} is that proteolytic activity of protease may fully or partially deactivate other enzymes. The active sites of the enzymes may be affected. At those active sites protease may donated or accepted hydrogens, and destabilized the electrical charge build-up along the reaction mechanisms (Shafee, 2014).

Smaller particles of a powder material may result in lower resistance to flow. During densification and compaction processes, an interaction between enzymes and microalgal particles can influence lower resistance of a powder matrix in the die. At elevated and controlled temperature of the die this is even easier to control. Rheological properties of pre-compacting powders change due to enzymatic hydrolyses and chemical changes of the total protein (Table 2). The availability of protein bound to phytate, can be increased noticeably by hydrolysis of the phytate molecule by phytase. This agrees with Kies et al. (2001). Such changes may further influence the compacting properties of the system and lower p_{max}. This all may result in the decreased consumption of electricity during commercial pelleting. Lowering p_{max} during commercial pelleting and thus reducing retention time of the material in the pellet-die may increase the overall production capacity during pellet manufacturing. In a commercial microalgal biomass pelleting, the main concern is the ability of the powder material being pressed through the pelleting die. If the viscosity of material is high the increased temperature in the die will be generated by friction between the compacted powder and the die. Such temperature increase may cause burning of the compacted material and hence destruction of the nutrients. Mechanisms responsible for inter-particle reactions, through random distribution, are the rearrangement of particles in the clusters. This cluster-alike arrangement helps particles gaining the conformational entropy and the final stability of compacted powder materials (Pietsch, 1991; Walstra, 2003). Every system changes its volume when it is loaded. In other words, when particles of the microalgal biomass are loaded, they will have a strain response. Due to enzymatic reactions between phytase and protein linked to phosphorous, the enthalpies and the entropies could change their energy values and the differences in the p_{max} values. This agrees with Miladinovic and Salas-Bringas (2014). Using xylanase and phytase may possibly influence the reduction of electrical energy consumption during commercial pelleting, where non-starch polymers and microalgal biomass will be included. Further work related to the influence of enzymes must be done on the commercial scale and with direct measurement of electrical consumption, with clamp-on measurement devices.

With or without applied stress to all particles in compacted microalgal biomass, their macromolecules and atoms are subject to constant forces between them. In the present study, enzymes neither influenced the inter-particle bonding (hardness) within the pelleted microalgal biomass, nor did the a_w changed in the pellets. Thus, it can be assumed that no chemical change involving enzymes influenced change in the entropy of the compacted particles at the level of pellet hardness. It seems that the molecules or the chemical groups in microalgal biomass compacts were not modified to produce changes in the strength of the pellets. The results did not reveal any changes regardless of dosage of enzymes. Reason for this may be non-sufficient water content in the microalgal biomass for fully functioning enzymes. The bulk powder, made of microalgal particles, may have partitioned the hydration water from the enzymatic solution. This is highly dependent on the polarity of organic solvents (Yang et al., 2004). In this way the active surface of the enzyme is not well hydrated to make any change at the surface of the particles. so that particles of the microalgal biomass could interlock and create harder pellets. This finding may be explained by some other factors influencing the observed correlation outputs, and thus, further investigation is necessary.

Particle detachment was observed when oil droplet was placed on the surface of the pellet. Statistical differences in θ could be explained by different interparticle distances in the compacts, which increased as the oil droplet was ageing. These results should not be interpreted as the enzymes did not affect the θ at a higher percentage of enzyme addition, which may be beyond the quantities used in this experimental work. These differences in θ may be explained by the activation entropy of the protein and the chemical components of the microalgal cell walls at processing temperatures where hydrophobic bonds may become weak (Liu et al., 2000). Therefore, the particles in compacts detached more quickly when oil was placed on them, thus increasing lipophilicity.

The amount of water added to the microalgal biomass was close to that in the commercial pelleting process. A low a_w indicates a low activity of enzymes. Lee and Kim (1995) explained that the enzyme reaction rate at a given water activity is different and that the reaction rate can be dependent on the property of the solvent. Enzymes need water to react and thus, a low a_w can indicate that the enzymes in the present experiment may not have reacted completely. The commercial pelleting process involves the moisture addition by steam conditioning. Such conditioning does not provide an adequate water content but provides the high enthalpy to the powder material. In such way the "nearly full" potential of the enzymes may be achieved prior to pelleting. The full potential of the enzymes may be accomplished with processes that include sufficient water content, such as extrusion technology. Decreased a_w of the microalgal biomass with addition of single enzymes at all three levels, can be explained by the enzyme trapping water between the particles. When enzymes were mixed and added to microalgal biomass, such change did not occur. This may be explained by proteolytic weakening of the function of other enzymes by protease. Additionally, it was expected that free water decreased friction between the compacted medium and the compaction die-wall, hence, decreased p_{max} values were expected. This did not occur.

The three enzymes, and their various dosages influenced the correlation between a_w and tensile strength of pelleted microalgal biomass. This could be explained by reduced adhesion of microalgal particles due to bound water presence. This was probably due to the free energy at the surface of the particles. This corresponds to the results obtained by Sun (2008). A significant positive relationship between a_w and tensile strength could be explained due to preferable water dynamics through hydrogen ionization, which occurs with the enzymatic reaction.

As hypothesized in this research, it has been shown that the UPS of the microalgal compacts can be positively influenced by adding any enzyme alone. This can be explained by the ability of enzymes to interact with a microalgal substrate, its disintegration and consequently systematic molecular packing and bonding of particles in the substrate. The overall tendency of the enzymes when added alone, independent of their dosage level, was to slow down the UPS process. However, the different effect of the protease/phytase on UPS at different dosages is still unclear. When all enzymes were mixed together in their double dosage, a lower UPS was observed. The complexity of such an enzymatic mixture may have allowed non-starch polymers, proteins and available phosphorous

to interact with each other, allowing the formation of strong molecular bonds. When all three enzymes were added together as the double dosage, most likely, xylanase helped hydrolysing the complex chain of polysaccharides and thus, increased hydrogen bonding between the molecules. When such a structure was formed, the double dosage of protease and phytase may have improved protein solubility, as explained by Bae et al. (2013). This study indicates that addition of enzymes is necessary to control the UPS, which could improve the environmental situation at the aquatic farms. The addition of the enzymes in such way may control the leakage of nutrients in the water. Which could help better situation in the aquatic farm.

5. Conclusions

Adding of the enzyme xylanase at recommended level or the enzyme phytase at all three levels reduced the resistance of microalgal pellets during discharge from a pelleting die. These enzymes could therefore possibly help to lower the electrical energy consumption during the pelleting process due to reduced friction between the pellets and the wall of the pelleting die (lower p_{max}). Adding of these enzymes was neither influencing the tensile strength of the pellets, nor the surface contact angle between the microalgal pellets and water or oil droplets.

Addition of enzymes xylanase, phytase or protease alone, used for this experiment, lowered the water activity. This may probably lower the microbiological activity in the microalgal pellets and lead to increased shelf life of the pelleted product. The reduced water activity did not influence underwater swelling or the surface contact angle, neither for oil nor for water droplets. Reduced water activity was directly correlated with reduced flow resistance of the compacted material in the pelleting die during pellet discharge. This happened most likely due to decrease of frictional forces between the pellet surface and the wall of the pelleting die.

The physical quality characterization of novel feed ingredients, if well used and understood, may have a positive economic impact for feed manufacturers. This is central when focus is the inclusion of novel ingredients into the feed matrix.

6. Further studies

Suggestions for further research includes water sampling for nutrient leakage by performing UPS analyses from the pellets treated with different enzymes. added alone or in combination. Also, future studies are needed to characterize different single cell organisms and their interaction with various enzymes and to assess their process-ability, nutritional and physical quality parameters during and after compaction. The use of higher amounts of water is suggested in the future for improved enzymatic hydrolyses. Preferably, water could be added with saturated steam after mixing and prior pelleting. Better understanding of enzymatic influence on the physical quality of the microalgal compacts may be achieved with an elaborate experimental plan. Such plan may include different compacting pressures, different temperatures, water addition, and enzyme concentrations beyond the levels used in this article, where focus must be given to power consumption during pelleting, underwater pellet swelling, hardness and durability of the pellets. Enzymatic activity is critical to consider in future scientific studies and for the commercial feed production where determination of pellet quality including microalgae would be essential. This could lead to better utilization of the raw materials and an overall better farm economy.

Declarations

Author contribution statement

Dejan Dragan Miladinovic: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Trond Storebakken: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Odd Ivar Lekang: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Carlos Salas-Bringas: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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Fish meal replacement with torula yeast (*Cyberlindnera jadinii*) and enzymatic treatment can affect the flow resistance in the pelleting die and the physical properties of the feed pellets

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Abstract

Out of many novel feed ingredients, yeast has positioned itself among the important ones. The presented work explains that the replacement of the fishmeal with the yeast *Cyberlindnera jadinii* and adding a modest amount of enzymes reduces the resistance of the feed material in the pelleting die for the duration of feed pellet discharge. Also, the presented experimental work focuses on how the replacement of the fish meal with yeast treated with enzymes can affect physical pellet quality of the feed. The experiment 1 was designed to answer if a change in the flow resistance and possibly a change in physical pellet quality may be expected if fishmeal is replaced by yeast. In the second experiment, the aim was to study if the feed containing yeast and the addition of an enzymatic mixture, may affect the flow resistance and physical quality of the feed pellets. Flow resistance in the die showed to be significantly increased by adding enzymes to the feed with 20% yeast. Flow resistance in the pelleting die showed to be significantly increased when feed with 20% yeast was treated with enzymes. The tensile strength of pellets with 20% yeast significantly increased in both experiments. Pellets with 10%, 20%, and 100% yeast showed aquaphobic behaviour when compared to pellets with 0% yeast at 94 seconds of observation time. Pellets treated with enzymes having 10% and 20% yeast showed lipophobic behaviour. Enzymatic treatment showed to decrease underwater pellet swelling of the pellets with 10% and 20% yeast. The longitudinal surface roughness of the pellets containing yeast and treated with enzymes decreased.

Increasing the tensile strength of feed pellets can be achieved by adding 20% yeast instead of fish meal. Enzymatic hydrolyzes should be used minimally in the pelleted aquatic feed for lower underwater disintegration. When the enzymatic mixture was added pellets with 20% yeast had a lower surface roughness of the pellets.

Key words: pellets, enzyme, physical characterization, single die pelleting

34 # 1. Introduction

35 ## 1.1. Sustainable feed without fishmeal

36 The expansion of aquaculture requires increased quantities of alternative and sustainable protein
37 sources (Aslaksen et al., 2007). Fishmeal remains an important part of aquatic-feed diets and the
38 feed manufacturing industry is a substantial consumer of fishmeal (Tacon and Metian, 2015; Hua
39 et al, 2019; Malcorps et al., 2019). Therefore, this growing industry and research institutions try
40 to target the best possible alternative candidates to replace fishmeal (Froehlich et al., 2018;
41 Pelletier et al., 2018).
42 In the last decade, various yeast species gained attention as a good source of nutritionally valuable
43 raw materials for aquatic feeds (Langeland et al. 2014; Vidakovic et al., 2015; Huyben et al. 2017;
44 Vidakovic et al., 2019; Agboola et al. 2020). Yeasts have high nutritional value based on protein
45 with an optimal composition of amino acids for farmed aquatic animals such as tilapia (Olvera
46 Novoa et al. 2002), salmon (Øverland et al. 2013) and shrimp (Gamboa-Delgado et al. 2015).
47 Yeasts can be successfully used as alternatives to fishmeal or soybean meal up to 24% without
48 impacting the growth (Guo et al., 2019) or health condition (Jin et al., 2018) of the aquatic animals.
49 However, replacing conventional feed ingredients with complete feed diets may influence the
50 physical quality of the feed. Loss of feed nutrients in the water may contribute to water pollution
51 (Tantikitti, 2014) and consequently increase stress on the farmed animals and reduce production
52 (Ferreira et al., 2011).
53 Generally, the acceptable physical properties of animal feed pellets are indicators of improved
54 feeding efficiency and convenient feed handling. Such evaluation may be done by characterizing
55 the influence of added novel material in the feed matrix on durability, hardness, the flow resistance
56 of the material in the pelleting die during pelleting, hydrophilicity, and pellet swelling underwater.
57 Evaluating the surface characteristics and hygroscopicity of the feed pellets containing a selected
58 alternative ingredient may help determine the functionality of that ingredient. This would further
59 contribute to better feed manufacturing, better shelf-life estimation, texture evaluation of the feed
60 product, and quality control (Stokes et al., 2013).

61 During the compaction of the feed mash, the resistance to the flow of the raw materials can
62 influence the consumption of electrical energy, appearance, consistency, and physical quality of
63 the final product. When feed material resists flow during pelleting the temperature increases in the

pelleting die. As the temperature elevates the sufficient water content in the feed mash being compressed may change the feed nutrients during processing (Hoseney et al., 1992). Controlled processing variables, such as the shearing of the material in the pelleting die, can alter the microstructure of the final product (Hermansson, 2000). Enzymatic treatment during feed manufacturing has a significant ability to better use feed ingredients for improved nutritional and physical quality of feed (Bedford and Partridge, 2011).

There is a lack of published data on how adding different enzymes relates to the flow resistance of the pellets through the pelleting die and the physical quality of the feed pellets. Lack of reported evidence of how the enzymes exo-endo-1,3-β-glucanase and protease affects the compacted feed material during pelleting. If the enzymes in a low water content environment are modifying the biochemical properties of the feed ingredients, this could lead to an altered flow of the material during compaction and thus modify the physical properties of the feed pellets. If the flowability changes due to changed pressure at incipient flow (p_{max}) in the pelleting die the power consumption of the pelleting equipment changes too. This can lead to a change in the physical properties of feed pellets. The entire spectra of different analyses may contribute to the overall understanding of the physical quality of pellets.

The objective of this work was to understand how the replacement of the fishmeal with torula yeast may influence the flow of the feed mash in the pelleting die with or without adding enzymes. Also, the aim was to show that replacing the fish meal with yeast and adding the enzymes protease and exo-endo 1-3-beta glucanase can lower p_{max} during pelleting. Lowering the p_{max} may modify the physical characteristics of feed pellets. Such modification may allow the feed fragments to pack and interlock better with other feed particles during densification and compaction processes. This may further contribute to harder feed pellets that can maintain their structure when being underwater and hence pellets can maintain longer their availability to be eaten by the farmed aquatic animal. The research represented by this work consisted of two experiments with single die pelleting as a model for processing conditions in commercial production. The first experiment was designed to give an indication of the optimal dosage of *Cyberlindnera jadinii* that may influence the physical quality of the pellets. The second experiment evaluated how the replacement of fishmeal with *Cyberlindnera jadinii* and the addition of enzymes may influence the physical quality of the final feed products.

2. Materials and Methods

2.1 Experiment 1

2.1.1 Raw Materials

All raw ingredients presented in Table 1, except *Cyberlindnera jadinii* and fishmeal, were obtained from the Center for Feed Technology, Norwegian University of Life Sciences, Ås, Norway. Inactivated *Cyberlindnera jadinii* yeast was obtained from Lallemand, Estonia in powder form with a dry matter of 970 g/kg, ash 78 g/kg, crude protein 470 g/kg, crude fat 16 g/kg, and gross energy 19.9 MJ/kg. Fishmeal was obtained from Norsildmel AS, Egersund, Norway with dry matter 917 g/kg, ash 145 g/kg, crude protein 684 g/kg, crude fat 73 g/kg, and gross energy of 19.4 MJ/kg. All materials used for the experimental diets, except *Cyberlindnera jadinii* and vitamin/mineral premix, were milled through a 1 mm screen size installed on an Alpine mill (model 160 UPZ, 1988, No. 13580.1). Yeast and vitamin/mineral premix were not milled due to their particle size below 1mm.

Table 1. Formulation of the experimental feed model presented as the % of the inclusion

2.1.2 Preparing the experimental feed

Mixing of the raw materials was done using a mixer Diosna (P1/6, Germany), with mixing tools based on three agitating paddles and a tulip-form knife. The speed of the paddles during mixing was 250 rpm. and the knife 500 rpm. During the mixing of the formulated diets, 10% of distilled water was added to the mixing mash with a spraying nozzle (Model 970, Düsen-Schlick GmbH, Germany). Water was added to integrate enough moisture for further processes. All diets were mixed thoroughly for 800 seconds. From each mixed diet, three samples were randomly taken and thereafter mixed to obtain a representative homogeneous sample. Representative samples were analyzed for moisture content in triplicate by the EU, No. 152/2009 method. The average moisture for all the trials was 13 % w/w. After moisture analyses were performed the mixed mash was vacuum-packed to avoid moisture loss. The samples were stored at -20 ºC for 3 days prior to steam conditioning and pelleting due to technical reasons related to assembling the single-die pelleting equipment.

2.1.3 Steam conditioning and pelleting

To ensure that every pellet will be similarly made the feed mash for each pellet was weighed out as 0.13 g and placed in the 30 Eppendorf tubes for each treatment. Thereafter all closed Eppendorf tubes for the specific feed were placed into the boiling water. The feed was conditioned for 3 minutes in boiling water to allow the water in the feed mash to become steam. Subsequently, the Eppendorf tubes were placed in the fridge at 4°C to cool down for 20 minutes before pelleting so that water can condense swiftly within the entire feed mash in the Eppendorf tube after conditioning. The single-die pelleting method described by Salas-Bringas (2010) was used for the compaction of the mixed feed into cylinder-shaped pellets. Steam-conditioned feed material from each Eppendorf tube was used to produce one pellet by pouring it into an 81 ºC preheated blank die channel. The temperature setting of the pelleting die is advised due to the elimination of possible salmonella contamination in accordance with Norwegian law (VKM, 2006). Poured feed mash was heated for 3 minutes before compaction to ensure an equal temperature of the mash particles. Pelleting was done with a compression rod having a diameter of 5.45 mm. The same temperature was used during pelleting and during the extraction of the pellet from the die. During pelleting, an initial pre-load pressure of 240 kPa was used. The setting for a maximal force load for each pellet was 285 N and applied compressibility was 12 MPa. The selected compacting force load was chosen according to the densities of commercial animal feed pellets produced on ring-die pellet press. This is explained in detail by Salas-Bringas et al. (2011) and Salas-Bringas et al. (2015). The compaction was done at a speed of 10 mm/min with a compression rod inserted in a 5.5 mm die channel with a closed end. Successively after reaching the maximal force load, the closed part of the die was removed. Subsequently, the pellet was discharged from the pelleting die at a pace of 2 mm/min. The selected pace was set to avoid getting beyond the compacting forces and thus avoid additional compaction. The total time of the material retaining in the pelleting channel was 9 minutes. Compacted pellets were 5.5 mm in diameter and had about 0.1 grams of weight. The loss of weight was due to high pressures and temperatures causing moisture evaporation. Each pellet was stored at 4 ºC in its Eppendorf tube prior to further analysis.

2.2 Experiment 2

2.2.1 Preparing the experimental mixes and sampling

Remained mixed feed from experiment 1 that was vacuum packed and stored at a temperature of -20°C was used. Vacuum-packed feed was taken out from the freezer and left at room temperature

overnight. Each diet was thereafter poured separately into mixer (Diosna P1/6, Germany). Prior to mixing, the enzymatic cocktail was prepared with buffer solution and enzymes, non-commercial protease (AB Vista, Marlborough, UK) derived from *Fusarium equiseti* and endo-exo 1,3-β-glucanase produced by Megazyme, Ireland and derived from *Trichoderma sp.*. Pre-prepared 100 mmol buffer solution, stored overnight at 4°C, was used as a carrier for the enzymes. About 0.9 ml of protease and 0.75 ml of endo-exo 1,3-β-glucanase were added to 15 ml of buffer solution. The pH of the mixture was 5.8, which was close to the producer's recommendation of pH 5.5 for both enzymatic products. The enzymatic cocktail of 16.65 ml was sprayed on the batch of 150 g for each feed mixture. The control diet was sprayed with 16.65 ml of distilled water without enzymes. Distilled water was used to avoid adding any impurities to the feed mash. Spraying was done with a spraying nozzle (Model 970, Düsen-Schlick GmbH, Germany) built into the mixer (Diosna P1/6, Germany). During the spraying of the enzymatic solution, the mixer was operated with the speed of the paddles set at 250 rpm. The speed of the knives was 500 rpm. All diets were mixed thoroughly for 800 seconds.

From each mixed diet, three samples were randomly taken and thereafter mixed to obtain a representative homogeneous sample. The representative samples were used to analyze moisture content in triplicate for each enzymatically enriched feed mash (EU method No. 152/2009). The average moisture for all feed diets was 15 % w/w. The rest of the treated feed mash was taken out from the mixer, vacuum packed, and stored at -20 ºC, until steam conditioning and pelleting to preserve moisture content. Steam conditioning and pelleting were done in the same way as in experiment 1.

3. Analytical techniques and measurements

3.1 Particle size distribution

The laser diffraction method was used to verify the particle size distribution of the feed-mixed mash. The device used for measuring particle size distribution was Mastersizer S instrument (Malvern Instruments Ltd, Worcestershire, UK). Calculating the volumetric particle size distribution of the light energy on the detector was done by deflecting the light based on the theory for spherical particles (Allen, 1997).

3.2 Measurement of compaction, pellet discharge, and pressure at initial flow (p_{max})

Maximum compaction pressure (Pa) at maximum force (N) during densification, compaction, and pellet ejection from the die were observed and recorded. The recording was done with NEXIGEN Plus software connected to a Lloyd texture analyzer (LR 5K Plus; Lloyd Instruments, U.K.). Measuring maximum compaction pressure at maximum force may point to possible alterations in electrical energy consumption connected to alterations in the resistance of the feed material to move through the die (p_{max}). The p_{max} was identified as the pressure needed to initiate pellet ejection from the pelleting die when the close end from the die channel was removed. Measurements were done as a flow resistance indicator to measure variations between trials, caused by friction on the contact area between the die and compacted pellet. The initial movement of the pellets through the die was recorded as a force needed for the pellet to start moving through the open 5.5 mm die hole. Measurements were done after compaction and when the blank part of the die was removed. The pellet discharge speed was set to 2 mm/min to guarantee that the pressure would be under the previous compaction pressures. The analytical results for maximal pressure before the pellet started moving through the die, were obtained using a Lloyd LR 5K Plus texture analyzer and assessed with Eq. 1.

$$p_{max} = F / \pi r^2 \quad \text{Eq. 1}$$

Where:

F – Load force (Nm)

r - radius of a pellet (mm)

3.3 Tensile strength

Results of the tensile strength analyses were done by applying maximum force (F) on the compacted feed pellet under diametral compression. Three randomly chosen pellets for each treatment were used. Tensile strength was measured for each pellet by recording the first peak force (F) while compressing the pellet across the diameter at a speed of 1 mm/min^{-}. The stress (σ) was estimated by using Eq. 2, well-known as the Brazilian test. A probe with a surface of 60 mm in diameter was used to measure σ (Eq. 2) while being connected to a Lloyd LR 5K Plus texture analyzer (Lloyd Instruments, U.K.)

$$\sigma t = 2F/\pi Dl \quad \text{Eq. 2}$$

213 Where:

214 σt - maximum tensile strength (MPa)

215 F - load at fracture of the first peak force (N)

216 D - feed pellet diameter (mm)

217 l - feed pellet length (mm)

218 3.4 Measuring contact surface angle (CA)

219 The drops of oil and water were disposed from a dosing needle and placed at the diametral plain
220 surface of a feed pellet, defined as the upper plain being at the side of a compression rod during
221 pelleting. The analyses of a liquid drop from a dosing needle, defined as the surface contact angle,
222 outline the surface energy between the adhering liquid and the pellet surface. Surface wetting of
223 the feed pellets, the effect of added yeast, and the enzyme treatment on CA for both oil and water
224 were evaluated with a video-based device OCA 15EC (Data Physics Instruments GmbH,
225 Germany) (Fig. 1). The CA analyses were completed on three randomly chosen pellets to compare
226 how liquid sorption of the pellets with rape seed oil or distilled water could be affected by the
227 experimental factors. The drop volume for distilled water was 1 μl and for rapeseed oil 5 μl. Due
228 to the larger surface tension of rapeseed oil, it was more difficult to detach the oil drop from the
229 needle, thus a larger drop volume was needed. The recorded drop absorption is measured in
230 seconds and presented in the results as the initial surface angle (T0) and final droplet age. Rapeseed
231 oil CA and distilled water CA were recorded within 3 and 1.2 seconds, respectively. The time
232 frame was decided for both oil and water droplets by full penetration of the liquids at the upper
233 diametral plain surface of a feed pellet. The CA measurements were done at a temperature of 18
234 °C. The software SCA20 (Dataphysics instruments GmbH, Germany) was used to record the
235 change of CA at different time intervals. When the initial CA was < 90°, the surface was considered
236 as hydrophilic, however, if θ > 90° a hydrophobic surface was considered, described by Förch et
237 al. (2009) and Mišljenović et al. (2015).

238

239 Figure 1. Setup for surface angle measurement. Letters indicating: A - video camera; B – light source; C – an image
240 of a drop on top of a pellet surface; D – dosing syringe. Adopted from Mišljenović et al. (2015).

241 3.5 Underwater pellet swelling rate analyses (UPS)

242 The UPS measurements explain if increasing yeast content in the feed instead of fishmeal and
243 enzymatic treatments may influence physical changes of the pellets. Also, the UPS indicates pellet
244 cohesivity (particle detachment), linked to the swelling rate of the pellet. A special prearrangement
245 to monitor the swelling of the feed pellets underwater was used as described by Miladinovic et al.
246 (2021). The constructed system consisted of a video microscope (Microviper) and microscope lens
247 (Allen ¼") mounted on the optical tensiometer (OCA 15EC, Data Physics Instruments GmbH,
248 Germany) as shown in Fig. 2 was used for the UPS measurements. Optical monitoring of pellet
249 swelling was done in accordance with Ferreira and Rasband (2012) with the image processing
250 software Fiji. Distilled water at a temperature of 20 °C was added to the experimental glass
251 container. For each treatment, four randomly chosen pellets were used for measuring UPS. The
252 reference of a starting point for UPS measurement was a cross-sectional view of the feed pellet
253 with a diameter of 5.5 mm. An image of a cross-sectional view of the feed pellet was taken every
254 60 seconds for an observation time of 40 minutes. The length of the observation time was chosen
255 as the time required for some aquatic animals to consume the pellet in the open farming system
256 (Lovell, 1998). The observation area defining the cross-section of a swollen feed pellet underwater
257 during observation time implies the UPS results.

258

259 Figure 2 - Full setup instrument for the UPS measurements (1 - Krüss Tensiometer; 2 - Water glass container with the
260 pellet; 3 - Allen zoom compact video microscope lenses; 4 - Microviper portable computer)

261 3.6 Surface roughness analyses

262 Surface roughness analyses were done to understand if the replacement of the fish meal with yeast
263 or the addition of enzymes may influence any changes to particle packing and thus surface-change
264 of the pellets. Results are represented by the irregularities existing on the surface of the feed pellets
265 in the diametral and longitudinal directions. The diametral surface roughness has been created by
266 interactions between feed particles during compaction. However, longitudinal surface roughness
267 is created as the interaction between particles and the die wall. Surface roughness is further
268 presented as a numerical scale of the surface condition influenced by novel materials, enzymes,
269 and diverse packing-ability of the particles in the pellets. The analyses were done with a surface

roughness tester (Surftest SJ-210, Mitutoyo, Japan).

3.7 Data analyses

The software used for statistical analyses was Minitab v.17 and for plotting the figures was used Microsoft Excel. ANOVA analyses were used to examine the possible effects of the increased percentage of yeast and enzyme addition. Tukey–Kramer method, using a 95 % confidence interval, was used to show significant differences between treatments. Pearson correlation test with a 95 % confidence interval was used to analyze correlations between variables.

4. Results

4.1 Experiment 1 – replacement of the fish meal with torula yeast

4.1.1 Particle size of the mash prior to compaction

All diets with yeast inclusion had similar particle size distribution, where 90% of the particles were between 400 μm and 500 μm. However, 50% of the particles were found to be between 80 and 120 μm with a significant decrease when adding more yeast to the feed. The same was observed with particles smaller than 20 μm that were only represented with 10% (Fig. 3).

Figure 3. Particle size distribution of the shrimp feed diets. Data representing means based on two replications. Different sequential letters (A, B, C, D, E) from ANOVA-Tukey statistical method indicate differences at 5% level

4.1.2 Flow resistance in the die during discharge of the shrimp feed pellet (p_{max})

The p_{max} results presented in Table 2 do not show any distinctive change when yeast is added and compared to control feed without added yeast. However, pelleting only yeast as a negative control, increased p_{max} over 17 folds compared to all other diets ($p<0.05$). Adding from 2.5% to 20% yeast in the feed did not change p_{max}.

4.1.3 Tensile strength of pellets

The hardness of pellets measured as tensile stress increased significantly ($p<0.05$) by including 20% yeast in the feed when compared to the control diet and the diets with 2.5% and 5% yeast. Pelleting only yeast without other ingredients showed increased hardness of the pellets by over 9 folds (Table 2). Tensile strength showed to be moderately correlated to p_{max} (Fig. 4), where almost

299 half of the tensile strength as a dependent variable may be explained by flow resistance during
300 discharge of the shrimp feed pellet from the die ($R^2 = 0.49$).

301

302 Figure 4. Correlation between p_{max} and tensile strength in experiment 1 ($p<0.001$)

303 4.1.4 Surface contact angle (CA) measurements for oil and water

304 By adding 2.5% and up to 20% yeast in the feed, the CA of the oil at zero time (T0) was not
305 different from that of the control feed. A difference at T0 for oil CA was, however, observed
306 between 100% yeast and 0% yeast ($p<0.05$). Pellets made of 100% yeast were more lipophobic as
307 compared to pellets with no added yeast. At 47 seconds the lipophilicity was more pronounced
308 ($p<0.05$) for feed pellets without yeast as compared to feed with 20% yeast and 100% yeast.
309 Similar results for oil CA were observed when pellets with no added yeast were compared with
310 10%, 20%, and 100% yeast at 94 seconds. Pellets with 10%, 20%, and 100% yeast at T0 showed
311 to have pronounced aquaphobic behavior when compared to control pellets without added yeast.
312 Similar effects were observed at 47 seconds. However, at 94 seconds the water CA showed to be
313 significantly lower ($p<0.01$) in the pellets with from 2.5% and up to 100% yeast (Table 2).

314 4.1.5 Underwater pellet swelling (UPS)

315 The UPS did not differ when control pellets (0% yeast) were compared to other pellets at the first
316 minute after submersion under water. Differences were observed between pellets with 2.5% and
317 100% yeast when measured at minute 1. Pellets containing 100% yeast tended to swell poorly at
318 the first minute. After 20 minutes the pellets with 20% yeast had swollen 1.8 folds and the pellets
319 with 100% yeast about 1.3 folds. Pellets with 100% yeast showed significantly ($p<0.05$) slower
320 swelling when compared to pellets with 20% added yeast (Table 2). UPS results showed having
321 low correlation to tensile strength ($R^2 = 0.3$) in experiment 1.

322 Table 2. Experiment 1. Results of p_{max}; tensile strength; oil and water contact angle (CA) and underwater pellet
323 swelling (UPS)

324

Data representing means and ±SD. Different sequential letters from ANOVA-Tukey statistical method signify the difference at 5% level. Presented p-values for p_{max} ($p<0.001$); a_w ($p>0.05$); Tensile strength ($p<0.001$); CA-oil ($p<0.05$); CA-water ($p<0.01$) and UPS ($p<0.05$)

4.1.6 Surface roughness at diametral and longitudinal direction

Irregularities at the surface of the pellets defined as surface roughness are presented in Fig. 5. Diametral surface roughness was different when yeast was added to the feed as compared to the control feed with no yeast. However, no significant change in surface roughness was observed for the diet with 5% yeast. Longitudinal roughness difference was observed only for the pellets with 20% yeast in comparison with the control pellets, and pellets with 2.5% or 100% yeast. A correlation between p_{max} and longitudinal surface roughness was observed. More than half of the longitudinal surface roughness, as a dependent variable, may be explained by flow resistance in the pelleting die during pellet discharge ($R^2 = 0.51$) (Fig. 6). However, the correlation between the diametral surface roughness and p_{max} was not observed ($R^2 = 0.07$).

Figure 5. Surface roughness of the pellets with no added enzymes for both diametral and longitudinal analyses. Data representing means based on 25 repetitions. Different sequential letters (A, B, C) from ANOVA-Tukey statistical method signify difference at a 5% level separately for diametral and for longitudinal measurements where p-values were lower than 0.001.

Fig. 6. Correlation between longitudinal surface roughness and p_{max}

4.2 Experiment 2 – Enzymatic treatment of the feed and its effect on physical pellet properties

4.2.1 Flow resistance in the die during discharge of the feed pellet (p_{max})

By adding enzymes in the feed with the inclusion of up to 5% yeast, no change in p_{max} was detected. However, when enzymes were included in the feed with 10% and 20% yeast a significant increase in p_{max} was identified ($p<0.001$) when compared to the control feed with added enzymes (Table 3).

4.2.2 Tensile strength of pellets

The measured hardness of pellets as a tensile strength showed to be significantly increased in feed with 20% yeast and added enzymes ($p<0.001$). This was not observed for other yeast-containing pellets with added enzymes when compared to the control feed (Table 3). It was observed that p_{max} had a very strong influence on the tensile strength of the pellets (Fig. 7).

Figure 7. Correlation between p_{max} and tensile strength in experiment 2 ($p<0.001$)

4.2.3 Surface contact angle (CA) measurements for oil and water

At zero time (T0), pellets with 10% and 20% yeast and added enzymes showed a lipophobic behavior ($p<0.01$) when compared to other treatments (Table 3, Fig. 8). After 5 seconds the lipophobic behavior was also observed at the surface of enzymatically treated pellets containing 5% yeast. At 10 seconds, this was also seen for enzymatically treated pellets containing 0% and 2.5% yeast. A similar response was observed for the rest of the analytical time, i.e. after 15 and 20 seconds (Table 3).

Figure 8. Change of contact angle (CA) from oil drop on the pellet surface at different time intervals. The curved line symbolizes the initial oil drop profile.

CA of the water drop placed at the pellet surface measured every 0.5 seconds for a total measuring time of 2 seconds, showed similar results for all feed diets at time zero. However, after 0.5 seconds the enzymatically treated pellet with 5% yeast had a significantly lower contact angle when compared to control feed without enzymes and pellets treated with enzymes containing 20% yeast (Table 2, Fig. 9). Similar observations were seen after 1, 1.5 and 2 seconds of CA measurements.

Figure 9. Change of CA from water drop on the pellet surface at different time intervals. The curved line symbolizes the initial water drop profile.

Table 3. Experiment 2: Results of p_{max}; tensile strength; oil and water contact angle; and underwater pellet swelling (UPS)

Data representing means and ±SD. Different letters (a, b, c, d) from ANOVA-Tukey statistical method indicate significant differences at 5% level. Presented p-values for p_{max} ($p<0.01$); a_w ($p<0.05$); Tensile strength ($p<0.001$); CA oil ($p<0.01$); CA water ($p<0.01$) and UPS ($p<0.01$)

4.2.4 Underwater pellet swelling rate (UPS)

The UPS analyses showed that by adding enzymes to the control feed without yeast there was a significant increase in pellet swelling one minute after submersion in water. After 40 minutes, the control feed with and without added enzymes was equally swollen. Conversely, the inclusion of 10% and 20% of yeast significantly decreased the UPS when compared to the enzymatically treated control feed ($p=0.003$) (Table 3). A similar trend was observed after 20 and 40 minutes after submersion in stagnant water. The UPS linearly decreased by increasing yeast addition in the feed, indicating that the addition of 20% yeast may result in a UPS decrease of about 31%. The UPS of the feed pellets showed to be independent of the tensile strength ($R^2 = 0.24$).

4.2.5 Surface roughness at the diametral and longitudinal direction of pellets treated with enzymes

Irregularities at the surface of pellets compressed with the same force are defined in this work as surface roughness. Fig. 10 shows that longitudinal surface roughness presented as the interaction between particles and the pelleting die wall is lowered in feed pellets treated with enzymes and containing yeast from 5% and up to 20%. The surface roughness results indicate a linear decrease of surface roughness along the longitudinal pellet wall by replacing fishmeal with yeast when the feed mash was treated with enzymes. However, for diametral surface roughness, created by interactions between feed particles during compaction, the same can be concluded only for 5% and 10% added yeast. No significant difference in diametral surface roughness between enzyme-treated feed containing 2.5% or 20% yeast.

Figure 10. Surface roughness analyses data representing means based on 25 repetitions each for diametral and longitudinal analyses. Different sequential letters from ANOVA-Tukey statistical method signify difference at 5% level separately for diametral and for longitudinal measurements where p-values were lower than 0.001.

The surface roughness of pellets with added enzymes was not influenced by p_{max}, either longitudinally ($R^2 = 0.08$) nor diametrically ($R^2 = 0.01$).

5. Discussion

5.1 Pressure at anticipant flow, p_{max}

p_{max} in the die during pellet discharge in experiment 1 showed to be independent of the addition from 2.5% up to 20% of the yeast *Cyberlindnera jadinii* (Table 2). When the yeast was the only medium to be pelleted, p_{max} showed to be significantly higher. This may be explained by changing particle size distribution of powder systems affecting their densification (relationship between particles) and compaction (relationship between particles and compaction die). Considering that the yeast particles are based on the deposited micro-scale material this could possibly diffuse from one particle to another through solid bridges during compaction. Thus, the forces influencing the cohesion of powder particles will rest on the contact area of the solid bridges and their diameter, which could all influence the build-up of a pellet with a high-density structure (Pietsch, 1991). A similar difference was observed in experiment 2, when the feed with 20% yeast was enzymatically treated. Diffusion of the powders with added enzymes caused a correlation between p_{max} and tensile strength.

5.2 Tensile strength

The effects of adding yeast to the feed and having an enzymatic treatment of the feed pellets with a limited amount of water showed to have an influence on the physical properties of feed pellets. The results indicate that the addition of up to 20% yeast in the feed led to a significant increase in pellet tensile strength in experiment 1. Furthermore, in experiment 2, the use of enzymatic hydrolysis with limited water and 20% yeast generally increased the hardness properties of the feed pellets. A possible explanation for these findings is the intense interactions and packing of the smallest particles (<100 μm) in the diet, which account for 60% of the particles. The interlocking bonds at the micro-scale between the small and larger particles may contribute to improved physical properties of the pellets. These particle relationships are influenced by factors such as geometrical arrangements, porosity, and the number of mono-sized particles. Densification, which refers to the rearrangement of particles within the contact areas under higher stress, can be influenced by minor changes in particle size and shape.

The interaction of the heterogeneous two-phase powder system depends on particle size, particle size distribution, and humidity. These factors can influence the displacement during pelleting and the diffusion of the powder systems. The diffusive or caging dynamics of the smallest particles within the system may play a role in these processes. The findings presented in this text align with previous studies. Barbarosa-Canovas et al. (2005) highlighted the importance of distribution on the physical properties of compacted products, while Yap et al. (2008) discussed the role of particle size and pore structure in the conversion of powders into solid structures. Additionally, Andersson et al. (2007) emphasized the influence of humidity on the dynamics of compacted powder systems.

5.3 Contact angle of oil and water

The measurements of oil and water contact angles showed a partial dependence on the observation time. The inclusion of 10% yeast led to a significant decrease in the oil contact angle. The time frame is an important parameter when adding yeast and enzymes in the feed designed to be fed to aquatic animals. Enzymatic hydrolysis and yeast inclusion decreased the contact angle for the oil drop. The results for water contact angle were less clear when the feed was treated with enzymes. The variations in contact angles can be explained by the density distribution of the initial powder and the packing of powder particles in the die. Korachkin and Gethin (2004) suggested that different packing arrangements of particles can create regions in compacted solids with differing densities during die-fill. Such variations in density can contribute to different surface contact angles for oil and water. The authors also observed different final densities in compacted solids due to varying vertical die-fill density distributions, which further supports the idea that density differences influence contact angles. The level of physical interaction between the feed pellets and different liquids in the presented research work appears to depend on the friction, adhesion, adsorption, and wettability of the surface. The findings from this work align with the work of Yuan and Lee (2013). The structural changes in the feed pellets caused by yeast and enzymes altered the properties of the pellet surface. These alterations can be attributed to the diffusion between particles and liquid absorption in the cavities present between particles, as explained by Roman-Gutierrez et al. (2003).

5.4 Underwater pellet swelling rate (UPS)

The disintegration of feed pellets underwater is considered a quality indicator of their usability by aquatic animals, as highlighted by Flemming (1995), Obaldo et al. (2002), and Bansemer et al.

(2015). The current research suggests that measuring the swelling rate of undisturbed feed pellets under stagnant water, as developed by Salas-Bringas et al. (2015) and applied by Miladinovic et al. (2021), is a valuable tool too. These measurements can indicate the optimal time duration that a pellet can retain its compact form before its consumption will start. UPS is ruled by the adsorption or desorption of the fluid by the physical and chemical properties of the compacted solids and their surfaces. The control treatments in both experiments, without yeast and without enzymatic treatment, exhibited 37.3% differences in UPS measurements during the initial 60 seconds, which can be attributed to a 2% difference in moisture content. Even insignificant differences in free water in the pellet structure facilitate the movement of water molecules through the porous structure of the pellets. Swelling of hygroscopic materials happens due to molecular adsorption in compacted feed solids. This molecular adsorption process involves bound water and capillary adsorption of free water. Free water molecules are attracted into the network of the compacted pellet structure through forces of attraction. If these forces are sufficiently strong, the water molecules are drawn into the structure, leading to an increase in volume. As the moisture content of the compacted solids increases, the molecular attraction decreases, resulting in further volume expansion. This volume increase corresponds closely to the volume of water added, and it leads to the deterioration of the feed pellets at a faster rate. Water equilibrium is reached when molecules start to move from the surface to the core of the pellets. In experiment 1, this equilibrium was achieved after 20 minutes, while in experiment 2, with the addition of enzymes, it was reached after 40 minutes. The replacement of fish meal with yeast in experiment 1 did not show any indication of influencing UPS measurements. However, in experiment 2, the addition of enzymes to yeast-free feed resulted in pellets being more prone to swell in the first 60 seconds. Enzymatically treated feed with 10% and 20% yeast exhibited significantly lower UPS measurements compared to enzymatically treated feed without yeast. These differences were consistent after 20 and 40 minutes as well. The presence of enzymes may have aided the formation of solid structures with yeast microparticles that had a low proportion of small cavities. These fissures within the pellets could facilitate water transport through capillary adsorption. Consequently, enzymes altered the water absorption ability of the compacted pellets, leading to a slower deterioration of the solids. This observation aligns with the significant increase in pellet tensile strength, which was more than tripled by the addition of 20% yeast.

5.5 Surface roughness

The observed surface roughness in the longitudinal direction varied in the pellets containing 20% yeast within pellets non-treated with enzymes. Such difference can be attributed to a higher number of smaller particles that were able to pack more effectively within the pellet, which aligns with the observed increase in tensile strength. The addition of enzymes contributed to a longitudinal decrease in roughness for feed containing 5%, 10%, and 20% yeast, but not for feed pellets having 2.5% yeast. The surface roughness of the pellets in the longitudinal direction was influenced by the particle size of the material, consistent with the findings of Sarkar et al. (2014) and correlated with p_{max} during pelleting. In this study, the addition of enzymes enhanced these effects.

6. Conclusions

The physical and technical quality characterization of novel feed ingredients integrated into feed pellets can have a positive impact on the physical quality. This is crucial when the focus is a dynamic change of the feed formulations. To avoid having high flow resistance in the die during pelleting the hydrolyses with enzymes should be avoided when having yeast included in the diet 10% and 20%. However, to increase the tensile strength of the pellets it is recommended to add enzymes to the feed with 20% yeast. Also, to decrease the pellet swelling underwater between 20 to 40 minutes the feed pellets containing 10% and 20% yeast should be enzymatically treated. Further research could explore additional variables and mechanisms involved in pellet formation to optimize feed pellet quality. Understanding these factors can contribute to the development of improved pellet formulations and manufacturing processes in the feed industry. Enzymes, in particular, contributed to the formation of solid structures with yeast microparticles, reducing the presence of small cavities. These findings provide insights into the mechanisms underlying the particle size/structure related properties of feed pellets.

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Tables

Table 1. Formulation of the experimental feed presented as the % of the inclusion

	Cyberlindnera jadinii (CJ) inclusion level (%)				
***Raw materials ID**	**0 CJ**	**2.5 CJ**	**5 CJ**	**10 CJ**	**20 CJ**
Wheat flour	30	30	30	30	30
Fishmeal	22.5	20	17.5	12,5	2.5
Soybean meal	10	10	10	10	10
Poultry meal	8.5	8.5	8.5	8.5	8.5
Rice flour	6	6	6	6	6
Soyprotein concentrate	6	6	6	6	6
Squid meal	5	5	5	5	5
Cyberlindnera jadinii	-	2.5	5	10	20
Monocalcium phosphate	1.6	1.6	1.6	1.6	1.6
Magnesium oxide	0.3	0.3	0.3	0.3	0.3
Mangandioxid	0.01	0.01	0.01	0.01	0.01
Monosodium phosphate	0.56	0.56	0.56	0.56	0.56
Vit/Min premix**	0.5	0.5	0.5	0.5	0.5
Water content (%)	9	9	9	9	9
TOTAL (%)	100	100	100	100	100

*Batch size 300 gram; **DSM - OVN™

531

532 Table 2. Experiment 1. Results of p_{max}; tensile strength; oil and water contact angle (CA) and underwater pellet swelling (UPS)

Added yeast	p-max (MPa)	Tensile strength (N/mm²)	CA - oil at 0 sec.	CA - oil at 47 sec.	CA - oil at 94 sec.	CA - water at 0 sec.	CA - water at 47 sec.	CA - water at 94 sec.	UPS (mm²) at 1 min.	UPS (mm²) at 20 min.
0 %	18.2^{b} ±6.0	18.1^{c} ±1.5	64.0^{a} ±2.0	62.9^{a} ±2.1	61.6^{a} ±2.2	63.1^{a} ±1.3	61.3^{a} ±1.5	59.5^{a} ±2.2	30.3^{ab} ±0.01	40.2^{ab} ±7.5
2.5%	16.6^{b} ±1.0	34.1^{c} ±14.8	63.3^{ab} ±1.5	60.7^{ab} ±1.3	58.8^{ab} ±1.0	59.7^{ab} ±4.4	53.2^{ab} ±5.1	47.9^{b} ±5.7	36.1^{a} ±1.8	40.8^{ab} ±3.5
5 %	17.4^{b} ±3.6	30.7^{c} ±3.1	57.5^{ab} ±10.1	54.2^{ab} ±10.7	57.4^{ab} ±4.9	59.1^{ab} ±5.1	49.3^{ab} ±6.5	43.7^{b} ±5.5	28.4^{ab} ±6.9	49.1^{ab} ±5.7
10 %	19.7^{b} ±4.1	44.2^{bc} ±6.6	61.2^{ab} ±3.0	59.3^{a} ±3.0	57.6^{bc} ±3.5	51.0^{b} ±3.8	44.0^{bc} ±4.5	40.1^{bc} ±3.4	33.0^{ab} ±2.1	47.2^{ab} ±10.8
20 %	23.9^{b} ±5.7	70.3^{b} ±5.0	50.7^{ab} ±1.2	49.6^{bc} ±2.1	48.7^{bc} ±2.7	49.9^{bc} ±5.7	44.9^{bc} ±4.7	42.5^{bc} ±4.6	28.8^{ab} ±0.8	51.0^{a} ±2.1
100 %	128.7^{a} ±43.1	173.8^{a} ±29.7	49.4^{b} ±7.0	48.1^{c} ±6.7	47.00^{c} ±6.4	38.3^{c} ±4.3	33.2^{c} ±3.5	31.0^{c} ±2.7	25.1^{b} ±0.5	32.8^{b} ±0.7

Data representing means and ±SD. Different alphabetical letters in superscripts from ANOVA-Tukey statistical method indicate significant difference at 5% level. Presented p-values for p-max ($p<0.001$); aW ($p>0.05$); Tensile strength ($p<0.001$); CA-oil ($p<0.05$); CA-water ($p<0.01$) and UPS ($p<0.05$)

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535 Table 3. Experiment 2. Results of p_{max}; tensile strength; oil and water contact angle (CA) and underwater pellet swelling (UPS)

Added yeast	p-max (MPa/mm²)	Tensile strength (N/mm²)	CA - oil at 0 sec.	CA - oil at 5 sec.	CA - oil at 10 sec.	CA - oil at 15 sec.	CA - oil at 20 sec.	CA - water at 0 sec.	CA - water at 0.5 sec.	CA - water at 1 sec.	CA - water at 1.5 sec.	CA - water at 2 sec.	UPS (mm²) at 1 min.	UPS (mm²) at 20 min.	UPS (mm²) at 40 min.
0% no enz	0.4^{bc} ±0.1	9.3^{bc} ±3.1	53.4^{a} ±0.5	42.2^{a} ±0.6	41.3^{a} ±0.6	38.5^{a} ±0.3	38.0^{a} ±0.3	53.1^{a} ±2.5	46.5^{a} ±3.0	40.4^{a} ±2.3	36.6^{a} ±2.1	33.2^{a} ±2.2	48.3^{b} ±6.8	80.5^{abc} ±6.3	106.5^{ab} ±5.0
0% + enz.	0.3^{c} ±0.08	6.3^{c} ±1.8	52.1^{ab} ±0.2	41.5^{a} ±0.6	37.7^{b} ±0.6	35.2^{b} ±0.3	31.7^{b} ±0.3	58.1^{a} ±1.1	45.4^{ab} ±1.2	37.9^{a} ±1.6	31.8^{ab} ±1.3	25.7^{ab} ±1.7	72.6^{a} ±2.5	109.6^{a} ±2.3	116.4^{a} ±2.1
2.5% + enz.	0.3^{bc} ±0.04	8.3^{bc} ±0.7	51.0^{b} ±0.5	41.4^{a} ±0.4	36.7^{c} ±0.6	33.9^{b} ±0.2	31.6^{bc} ±0.3	59.0^{a} ±3.1	39.7^{ab} ±1.9	32.2^{ab} ±3.1	26.7^{ab} ±4.3	22.3^{ab} ±5.0	62.5^{ab} ±9.6	100.3^{ab} ±12.2	105.2^{ab} ±7.6
5% + enz.	0.3^{bc} ±0.02	7.9^{c} ±0.6	52.1^{ab} ±0.3	35.5^{b} ±0.6	33.5^{c} ±0.6	30.7^{c} ±0.5	28.7^{d} ±0.4	56.2^{a} ±1.2	29.3^{b} ±2.2	19.2^{b} ±3.6	10.2^{b} ±5.9	4.6^{b} ±1.3	53.5^{ab} ±5.8	74.0^{abc} ±5.9	97.0^{abc} ±5.9
10% + enz.	0.5^{b} ±0.01	12.4^{b} ±0.3	45.9^{c} ±0.1	36.1^{b} ±0.6	32.7^{c} ±0.8	31.1^{c} ±0.2	29.7^{d} ±0.4	57.1^{a} ±0.9	35.3^{ab} ±1.7	30.6^{ab} ±2.1	27.1^{ab} ±2.4	24.5^{ab} ±2.7	37.3^{b} ±1.1	73.9^{bc} ±0.2	92.1^{bc} ±3.0
20% + enz.	0.8^{a} ±0.03	19.2^{a} ±0.7	46.4^{c} ±0.3	38.1^{b} ±0.6	35.1^{bc} ±0.6	32.0^{c} ±0.4	29.9^{cd} ±0.5	58.9^{a} ±4.4	49.7^{a} ±7.0	49.7^{a} ±7.0	42.1^{a} ±8.0	40.1^{a} ±8.1	36.7^{b} ±0.5	66.4^{c} ±1.1	80.3^{c} ±2.7

Data representing mean values and ±SD. Different alphabetical letters in superscripts from ANOVA-Tukey statistical method indicate significant difference at 5% level. p-values: p-max $p<0.001$; Tensile strength $p<0.001$; CA-oil $p<0.001$; CA-water 0' $p=0.6$; CA-water 0.5" $p=0.01$; CA-water 1" & 1.5" $p=0.007$; CA-water 2" $p=0.003$; UPS 1' and 40' $p=0.003$; UPS 20' $p=0.004$

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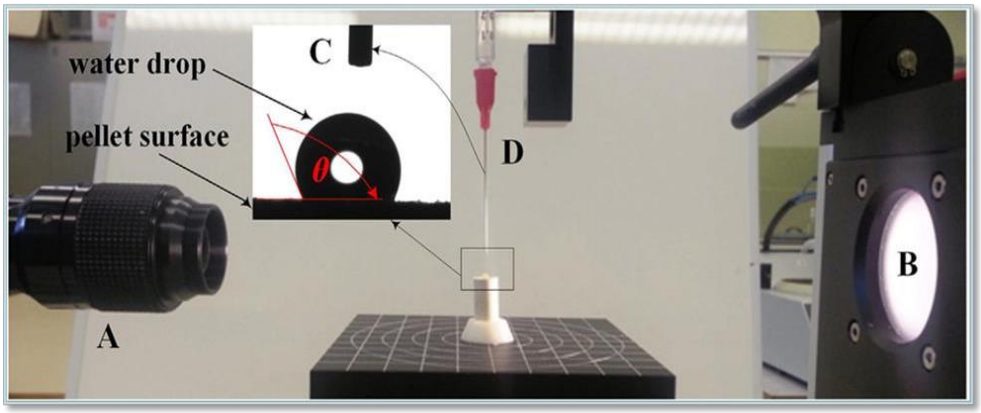
Figures



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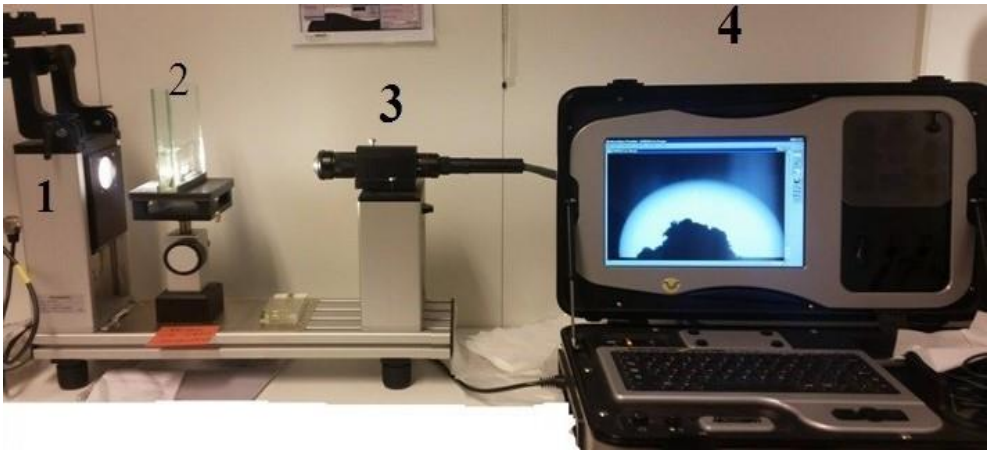

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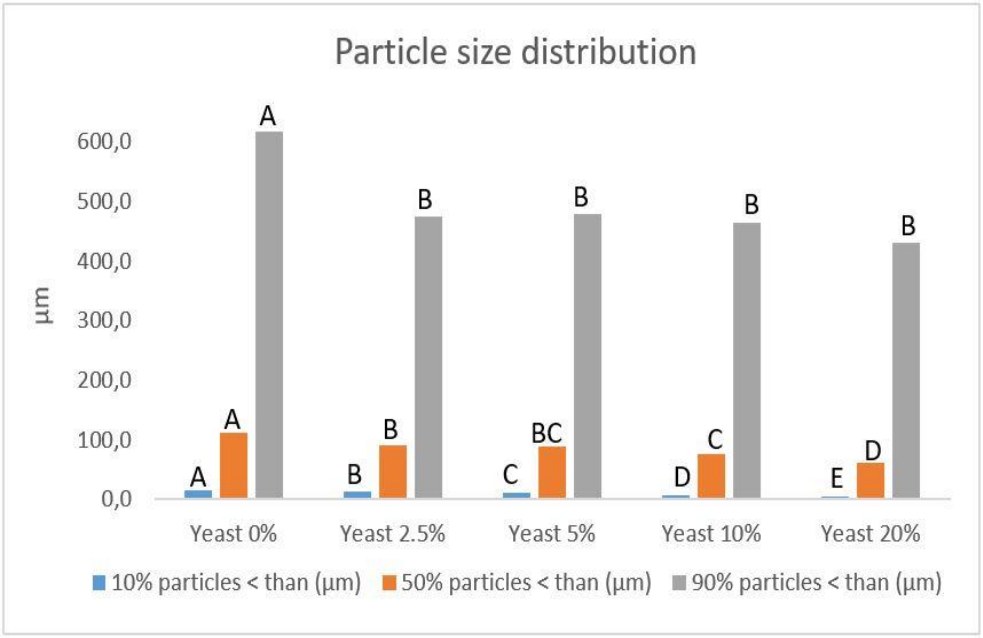

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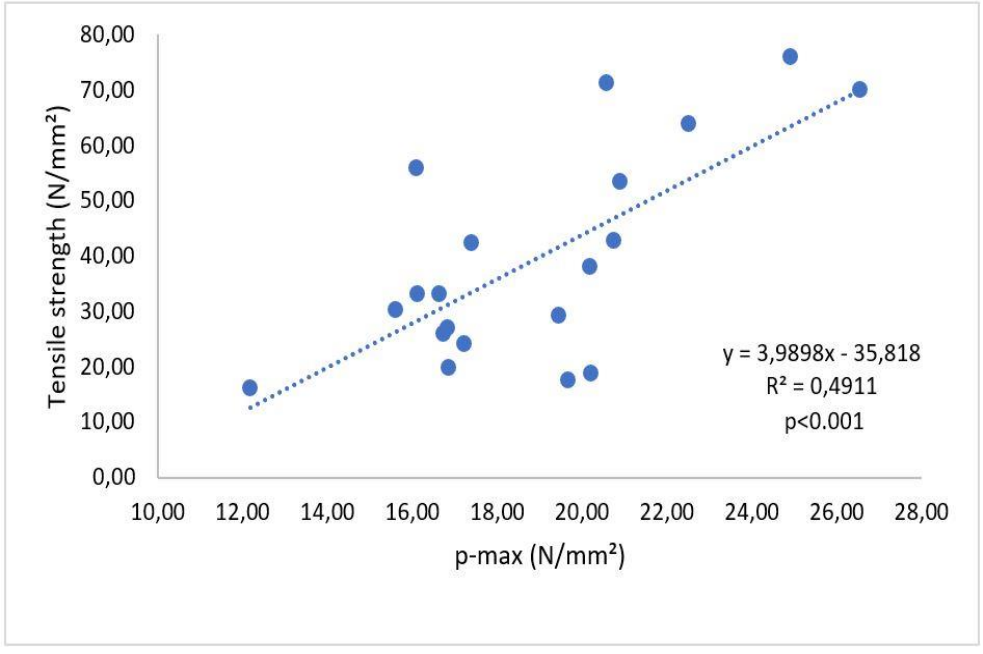

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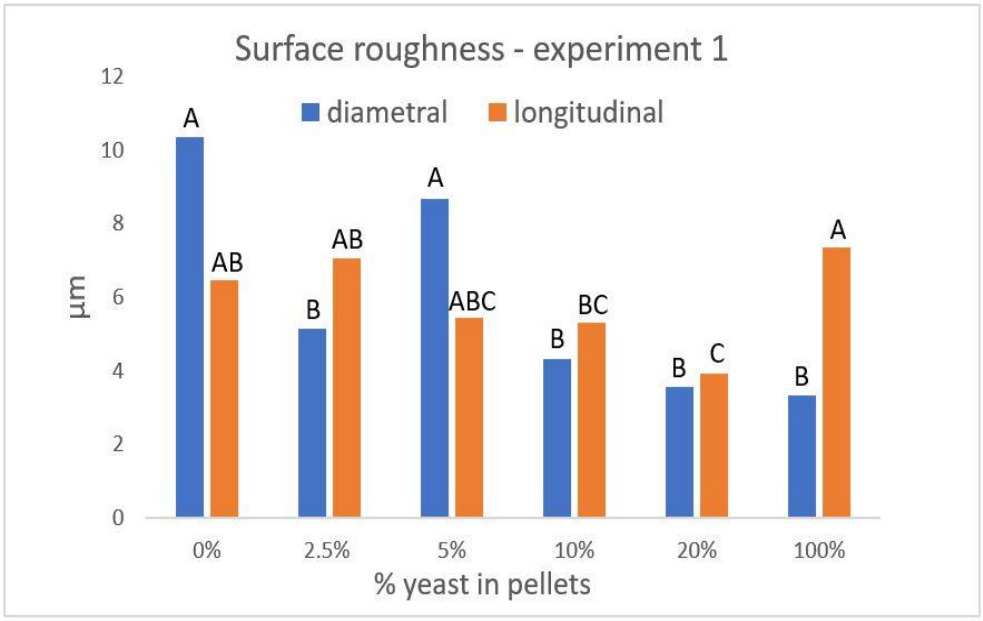

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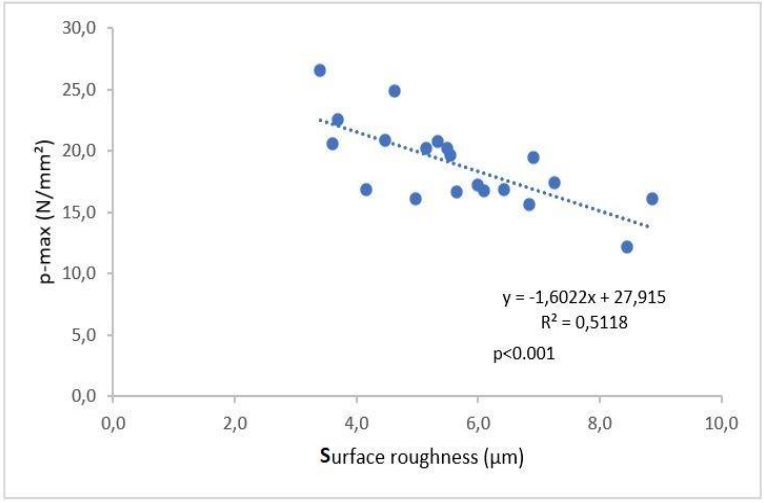

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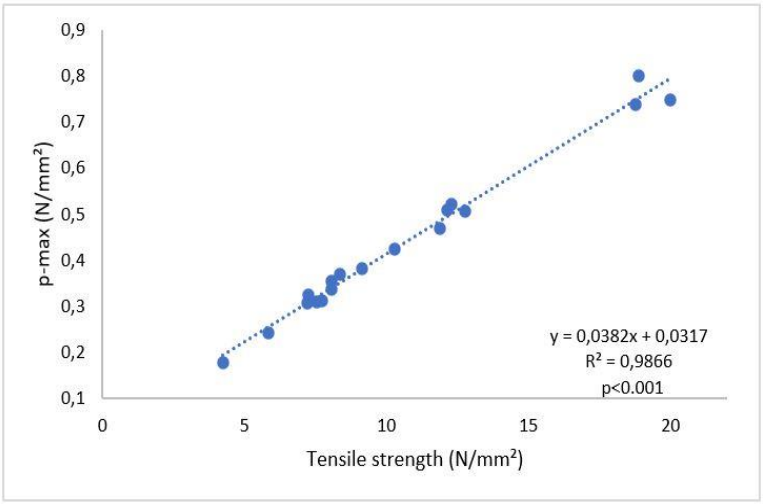

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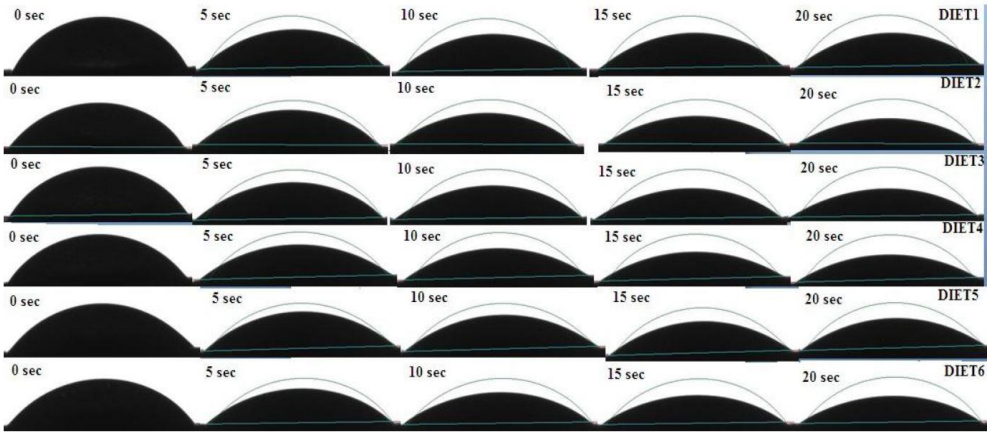

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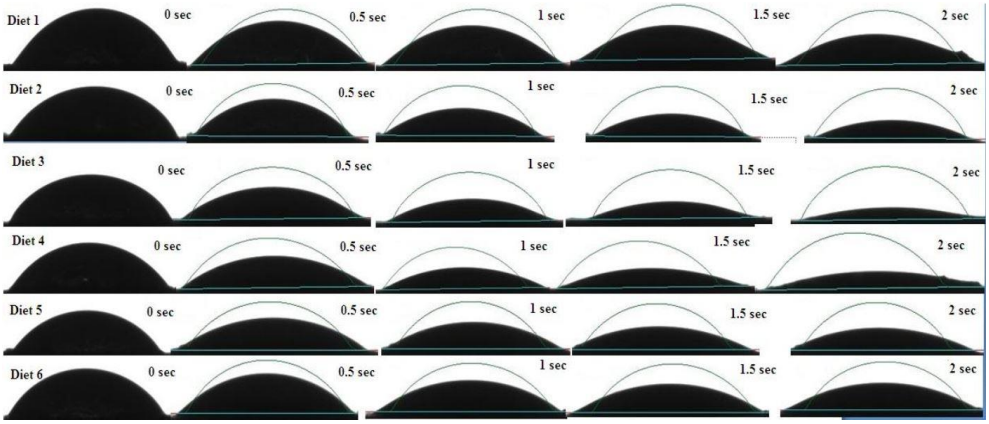

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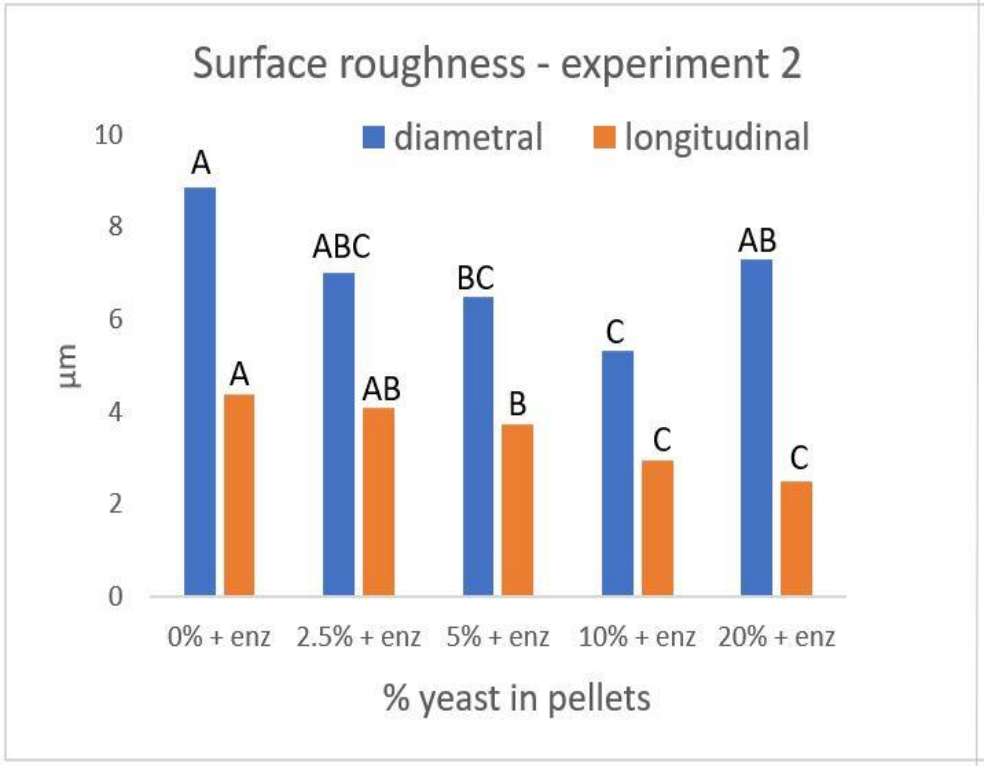

Figure 10.

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