Norwegian University of Life Sciences

**Master's Thesis 2020 30 ECTS**

Department of Animal and Aqua-cultural sciences
Faculty of Biosciences
Main supervisor: Dejan Dragan Miladinovic

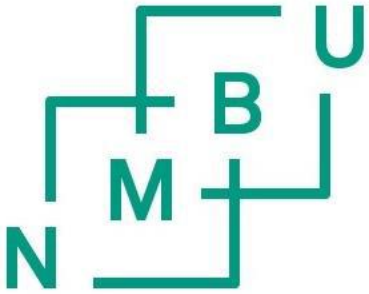
# CHARACTERIZATION OF BINDING ABILITY OF THE COMMERCIAL AND NOVEL FEED BINDERS IN STARCH AND PROTEIN BASED PARTICLES.

Christian SARPONG
Master of Science in Feed Manufacturing Technology
Faculty of Biosciences

# CHARACTERIZATION OF BINDING ABILITY OF THE COMMERCIAL AND NOVEL FEED BINDERS IN STARCH AND PROTEIN BASED PARTICLES.

**Department of Animal and Aqua-cultural Sciences**

**Faculty of Biosciences**




**Master thesis 2020**

**30 ECTS**

**Christian SARPONG**

**Master of Science in Feed Manufacturing Technology**

**Submission date: June 2nd, 2020**

# DEDICATION

I dedicate this work to my mother, Lydia Agyare. Without your endless support and motivation, thoughtful guidance, and genuine care, I would not have reached this far.

Also, to my father, Mr Samuel Sarpong and my siblings: Benjamin Opoku Sarpong, Joshua Sarpong and Comfort B. Sarpong. Brothers, thank you for supporting me in my education.

# ACKNOWLEDGEMENT

To God, be the glory for leading me where my trust is without borders.

I express my deepest gratitude to my supervisor, Dejan Dragan Miladinovic and Co-supervisor, Leszek Michalak, for their benefit guidance, useful discussions, and good comments during the work. May God, the creator of all things richly blesses you for helping me realise my potential.

I would also like to appreciate Stine Telneset, my academic advisor, for her excellent advice throughout the academic years.

My heartfelt gratitude to my dear parents and family members for their love advice, suggestions, consoling talks, and so on.

I especially thank my brother Joshua Sarpong for his unwavering support to me throughout my academic pursuit and assuming the role of a father in my life.

Finally, I thank all my friends for making my graduate life enjoyable.

# ABSTRACT

Pelletization aims to agglomerate a small particle of feed to become a larger particle using pressure, moisture, and heat. Pellets must have a basic form of physical quality in terms of hardness and durability to withstand the rigor of transportation. The objective of this work was to evaluate how spruce galactoglucomannan and its derivates may influence better binding ability between wheat-based particles (wheat flour, wheat starch and wheat protein concentrate). Also, to assess the acetylation level of the galactoglucomannan that function the best as a binder. Evaluating the effect of the galactoglucomannan on binding ability, guar gum (positive control (**B5**)) and lignoBond (negative control (**B6**)) were used as the reference binders. Diets were formulated with the feed binders at three different inclusion levels: 0.25%, 0.5% and 1.0%. Eighteen experimental diets were formulated with wheat flour, wheat starch, and wheat protein concentrates. Each experimental diet was made ten times. The pellets were made by using a single die pellet press method. Physical pellet quality parameter was analysed to determine the effect of the binders on p-max ($N/mm^2$), tensile strength/hardness (N/mm), water activity (aw) and moisture content (%). The effect of the test diets was compared to the control diets for wheat flour, wheat starch and wheat protein concentrate. For wheat flour, the test binders (diet with B1, B2, B3, and B4) showed a similar effect on P-max ($N/mm^2$) except B3 at level 0.25% and 0.5%. Again, P-max ($N/mm^2$) from wheat starch and wheat protein concentrate were generally similar with the exception of B2, B3 and B6 at levels 0.5, 1.0 and 1.0% respectively. Further, on wheat flour, the water activity (aw) results showed that at least the values of the test binders fall within the range of the positive and negative control binders' values. On wheat starch, at least one level of each of the binders had no significant effect compared to the control binders on water activity (aw). Though B2 at 0.25% and 0.5% recorded the highest water activity values. On the contrary to wheat protein concentrate, B3 affected water activity (aw) at all inclusion levels. Also, the p-values analysis showed that neither the binders nor the inclusion levels affected the tensile strength of wheat flour diet. For wheat starch, the analysis showed that the effect on tensile strength (N/mm) was the same among the binders except at B1, B2 and B3 at level 0.5% where a significant effect was observed compared to the others. Tensile strength (N/mm) from the wheat protein concentrate for the test binders were similar to both positive and negative controls except at B1 and B3 at 0.5% where a significant difference was observed with B6 at level 1.0%. The diets from wheat starch with B2 and B3 at all levels showed no significant effect on the moisture content compared to B5 and B6. The highest moisture content was observed at B4 level 1.0%, and it was

significantly different from B5 and B6. For wheat flour, a significant effect on moisture content was observed only between B5 and B6 at level 1.0%. The wheat protein concentrate diets results showed no significant effect among the test binders (B1, B2, B3) and B5 at all levels except B3 at level 1.0% where a significant difference was observed. But a significant effect was observed among the levels of B1, B2, B3 and B6, except at level 1.0% and 0.5% where B3 and B6 respectively showed no effect. Treatment B4 at all levels showed a significant difference among both B5 and B6 at all levels except at B5 level 0.25%.

**Keywords:** Pelleting, Pellet quality, Wheat flour, Wheat starch, Wheat protein concentrate, Galactoglucomannan, Guar gum, LignoBond, Pmax, Water activity, Tensile strength, Moisture content.

# Table of Contents

# List of tables

# List of figures

# Abbreviations

| | |
|---|---|
| PDI | Pellet durability index |
| GMD | Geometric mean diameter |
| µm | Micrometre |
| % | percentage |
| ℃ | Degree Celsius |
| ℉ | Degree Fahrenheit |
| N | Newton |
| KN | Kilonewton |
| Nm | Newton metre |
| $mm^2$ | millimetre square |
| g | gram |
| W | Watt |
| V | Volt |
| r | Radius |
| Pmax | Maximum pressure for pellet discharge |
| ANOVA | Analysis of variance |
| SE | Standard error |
| p | Probability |
| > / < | Greater than or less than |
| GGMs | Galactoglucomannans |
| B1, B2, B3, B4 | Test binders |
| B5 | Positive control binder (guar gum) |
| B6 | Negative control binder (LignoBond) |

| | |
|---|---|
| PaR5K | *Picea abies* Retentate from 5KDa filtration |
| NaOH | Sodium Hydroxide |
| CMC | Carboxymethyl cellulose |
| PMC | Polymethylolcarbamide |
| CAGR | Compound Annual Growth rate |
| USD | United State Dollar |
| FAOSTAT | Food and Agricultural Organisation Corporation Statistical Database |
| FAO-GCARD | Food and Agricultural Organisation – Global Conference of Agricultural Research for Development |

# CHAPTER 1. INTRODUCTION

## 1.1 Pelleting

Livestock and poultry feed come in many forms such as mash, pellet, and crumble. Mash typically causes some waste as a result of the separation of grains (particles) from other ingredients and supplements. This setback is solved in pellet feed that creates uniform proportion and improved digestion (www.bentoli.com, 2020). During the mid-1920s, the United States feed industry introduced pelleting to improve feed utilisation, increase the density of the feed and improve handling qualities (Gao *et al.,* 1999).

Pelleting of feed is a composite and delicate process which is dependent on several criteria including feed formulation, particle size, raw material abrasiveness, moisture, conditioning, fat or molasses levels added, roll settings, die maintenance and cooling temperature. From one feed to another, these criteria differ, so does the final pellet (dust) and homogeneity (nutrients) (Alliance machinery, 2012).

According to Subwilawan *et al.* (2019), pelletisation aims to agglomerate a small particle of feed to become a large particle using mechanical pressure, moisture, and heat. Also, Kaankuka *et al.* (2013) stated that the purpose of pelleting is to take a finely divided, sometimes dusty, unpalatable, and difficult-to-handle feed ingredient, and by using heat, moisture and pressure, form it into larger particles. The pellets are formed from wet mash that has already been mixed with all raw materials and compressed through the die. The early pelleting process involved mixing the feed ingredients and pelleting them with no further treatment (Gao et al., 1999). The rationale for this approach was to prevent alterations to vitamins and proteins due to the addition of heat to the feed mix. In the late 1930s, some processors began subjecting pellet-forming mixtures of animal feed to water and steam by passing the mixtures through a conditioner prior to introduction into the pellet extruders (Gao et al., 1999).

The conditioning process causes partial gelatinisation when steam is added to the mash (Sievert and Pomeranz, 1989). Moisture from steam makes starch with high carbohydrate content raw material swell while heat from steam tickers the gelation process to improve pellet durability (Subwilawan *et al.,* 2019). Skoch *et al*. (1981), reported that the use of steam improves pellet quality by reducing the amount of fines due to densification, hence increasing pellet durability. Also, the addition of steam reduced die wear and improve production rates (Gao *et al.,* 1999). Research has shown that providing pelleted feed to the animal improves feed intake, weight gain and feed conversion as compared to feeding a meal form of a diet

(Abdollahi *et al.*, 2019). As stated by Behnke (1994), the improvements in the performance of animals when fed with pelleted feed have been attributed to decreased feed wastage, decreased ingredient segregation, reduced selective feeding, less time and energy expended for prehension, destruction of pathogenic organism (by thermal treatment), thermal modification of starch and protein, improve palatability. Furthermore, feed pellets are much more precise and easier to control over the desire feed ration for individual animals or groups of animals with greater nutritional needs, such as immature stocks or lactating females (Mahapatra *et al.,* 2010, and Supriya *et al.,* 2012). Birds fed pelleted diet benefit from reduced energy use due to less time eating and digestion in comparison to those fed on mash feed (Mahmoud, 2017). This is because the pellet simply puts the feed in a concentrated form and preventing the animal from spending time to pick and choose between ingredients.

From the 1960s, the focus on research into pelleting was on improving the conditioning operation, with emphasis on increasing the retention time and increasing the temperature to which the mash is conditioned. In addition, more recent development was a pressure pelleting system in which the conditioner and pelleting die cavities were pressurized. This allowed the use of high temperature and longer conditioning time to improve pellet durability and increase the production rate. Later, a cold pelleting process was invented to use liquid binders in place of steam.

This present work considers the use of novel feed binders in the presence of steam to improve pellet quality. The binders are considered as ingredients and have cohesive properties. When applied to the feed ingredients, its binding characteristics aid agglomerate fine particles into large particles when compressing through a die. There are several additives, including guar gum and lignoBond, which are commercially used as binding agents in the feed industry. Their ability to bind feed particles together are well recognised and accepted in the feed industry; hence they served as benchmarks or reference points to the novel binders in this experiment.

The aim of the study: i) is to evaluate how the galactoglucomannan and its derivates may influence better binding ability between the wheat-based particles. ii) To assess the acetylation level of the galactoglucomannans that function the best as a binder.

# CHAPTER 2. LITERATURE REVIEW

## 2.1 Pellet quality

Pellet quality has been more critical in the poultry and swine industries as integrators continue to expand and identify the value of feeding high-quality pellets. This is because a well-pelleted feed has been found to enhance digestive capacity and thereby improve broiler live performance and feed efficiency (Amerah *et al.,* 2007; Abdollahi *et al.,* 2013). A good quality pellet can withstand repeated handling such as bagging, transportation and moving in feed line without excessive production of fine particles. Transportation and handling in both factory and on the farm require pellets of certain integrity without fines produced by attrition stresses (Thomas and van der Poel, 1996). A pellet of high physical quality must have properties which give high nutritional quality in terms of high feed intake and perhaps improved nutritional value (Skoch *et al.,* (1983); Stevens (1987))

Pellets need to have a basic form of physical quality in terms of hardness and durability to withstand the rigors of transportation (Thomas *et al.,* 1998). Hardness is the impact or pressure required to smash pellets at a time and the quantity of fines obtained from pellets after being exposed to mechanical treatment measures the durability. Pellet quality is mathematically expressed as the pellet durability index (PDI), and measured by using tumbling can device, Holmen pellet durability tester and tube tester (Winowiski, 1995). The pellet test sample is first sieved to separate fine, then keel over in the tumbling can device for a defined time (Mahmoud, 2017). The quantity of whole pellets is ascertained after the tumbled sampled in sieved to remove fines. Fines in feeders may result in feed wastage, animal refusals, and increased feeder management (Behnke, 2001).

Pfost (1963) stated that pellet quality parameter could be used to evaluate the effects of the diet formulation, conditioning, expander treatment, pellet binders, die selection etc. Understanding the essence for aggregating particles of a different size, shape and hardness is a key for optimisation of product quality in terms of physical properties. Hence, it is necessary to discern how feed ingredients are held together and to gain an accurate and deep understanding in binding mechanism and binding properties of pellet and behaviour when transporting and at storage.

### 2.1.1 Factors that influence pellet quality

#### *2.1.1.1 Feed ingredient/ feed formulation*

The least-cost formulation is designed to meet the nutritional parameters needed for the target animal (Behnke, 2001). It leads to many feedstuffs incorporated at different inclusion levels. This may result in variation in physical quality of the feeds after pelleting, although the calculated nutritional requirements are met (Thomas *et al.,* 1998). Additionally, some pelleting parameters, for instance, pressure (distribution) in the die hole and porosity, will change during pelleting. Also, the amount of energy required to overcome friction in the die hole can only be roughly estimated rather than exactly measured, and these parameters are strongly dependent on the physico-chemical properties of the diet ingredients themselves (Thomas *et al.,* 1998). Nevertheless, most nutritionists rarely think about the effect of formulation on processing, specifically pelleting. Different ingredients have different degree of pelleting ability and need different levels of steam conditioning to attain optimum gelatinization (Kenny and Rollin, 2007). Fat addition to the mash pre-pellet usually results in decreased pellet quality (Headly and Kershner, 1968; Richardson and Day, 1976). Moreover, the addition of protein and fibre materials enhance pellet quality (Behnke, 2001). McKee (1988) increased quality of pellet and water stability of catfish diets through increasing the level of wheat gluten from 0% to 10%. Lopez (1993) also reported that the addition of vital wheat gluten gave a positive effect on pellet quality and water stability, but a negative effect was observed by the addition of cassava meal.

#### *2.1.1.2 Particle size*

Reducing particle size from a coarse to a fine grind increases particle surface area per unit volume for the absorption of condensing steam and thereby increasing the number of contact sites among particles (Muramatsu *et al*., 2015). Lowe (2005) reported that the larger surface area of small particle sizes favours heat and moisture transference to the mash inside the conditioner. Variation in particle size results in a better pellet than a homogeneous particle size (MacBain, 1966). Though, intense reduction of particle size of feedstuffs may not be beneficial to pellet quality as proposed by (Muramatsu *et al*., 2015). Stevens (1987) confirmed that, when pelleting corn or wheat-based diets, the particle size had no effect on pellet durability index (PDI). Martin (1983) gave similar results using corn and grain sorghum. Also, Fahrenholz (2012) evaluated pelleted feeds formulated with corn of two different particle sizes (298 μm or 462 μm) and find no pellet durability index (PDI) differences. This lack of effect of the particle

size is possible since the evaluated geometric mean diameter (GMD) range was not enough to influence pellet quality. On the other hand, Wondra *et al.,* (1995) documented a pellet durability index increased from 78.8% to 86.4% when ingredient particle size was reduced from 1 μm to 400 μm. Hence, significant reductions in particle size may affect pellet quality.

#### *2.1.1.3 Water/moisture addition*

The water added to the mixer, and steam during steam conditioning helps pellet particle binding (Muramatsu *et al.*, 2015). The moisture in the feed, which is being processed in the conditioner serves as the medium to convey heat into the feed particles. Studies have shown that the addition of moisture to the meal has a positive effect on the conditioning process. Improvement in gelatinization can be achieved through proper moisture addition. The moisture addition and the improved pellet quality has been proven to improve the feed efficiency of broilers (Kenny and Rollin, 2007). Again, water acts as a lubricant and reduce the friction in the die, resulting in low bulk density and low energy consumption (Kaliyan, 2009). Although water has binding properties as well, but it has been proved that the use of steam over water is by far superior to produce good quality pellets (Thomas *et al.*, 1997).

The mash initial moisture content entering the conditioner is viewed to dictate the quantity of steam that can be added to the mash. Typically, not more than 6% moisture can be added at the conditioner (Leaver, 1988). Thus, large variations in initial moisture of mash will be reflected in the moisture of hot mash. This could result in different pellet mill performance if the quality of steam added to the mash are not regulated as the moisture changes.

High levels of heat and moisture are required to achieve proper pelleting, and since steam has a unique thermodynamic characteristic that allows for the transfer of heat and moisture simultaneously, steam conditioning is a necessary factor in pelleting (MacBain, 1966; Behnke, 2001). According to Reimer and Beggs (1993), the heat in conditioning is purposely to gelatinize the starch portion of the feed. Other benefits of heat are to destroy microorganism and to aid drying of pellets in the cooler.The moisture obtain from steam forms a cohesive bridge between particles and has a great effect on pelleting ( Smallman, 19996). To optimizing the process of conditioning, the proper balance of moisture and heat and must be attained. Steam has the potential to provide this combination; however, it exhibits different properties that must be understood and correctly utilized to produce high-quality pellets (Behnke, 2001).

### *2.1.1.4 Conditioning*

Conditioning is a critical step in the process of pelleting to obtain good physical quality. According to Yasothia (2018), the quality of conditioning process depends on the particle size of the mix, steam quality, initial moisture content of the mix, initial temperature of the meal as it enters the conditioner and the residence time in the conditioner. The conditioning process creates thermal, chemical, and mechanical energy. The steam used during conditioning breaks down the structure of starch, resulting in gelatinization also plasticizes proteins and softens fibres (Kenny and Rollin, 2007). Starch gelatinization in combination with protein plasticization enables binding among feed particles, and thereby it is important for the manufacturing of durable pellets (Behnke, 1994).

During steam conditioning, the mash requires saturated steam which consists mostly of vapor as opposed to wet steam which consists of free moisture. Wet steam conveys its heat less efficiently (thus, lower enthalpy of evaporation) than saturated steam and can result in uneven moisture distribution in the mash, causing choking or slipping of the pellet die (Kenny and Rollin, 2007). The quality of the steam affect the process of conditioning; saturated steam has been shown to increase mash temperature by 60 ºF (16℃) for every 1% added moisture, while wet steam increases mash temperature by 56 ºF (13.5℃) for each 1% increase in moisture (Kenny and Rollins, 2007). Steam quality is the percentage of saturated steam (vapor) in a saturated condensate (liquid)/ steam (vapor) mixture (Swagelokenergy.com, 2009). Simply, it is the amount of moisture in steam. A steam quality of 0 indicates 100 % liquid, (condensate) while a steam quality of 100 indicates 100 % steam. Poor steam quality can minimize conditioning temperatures by 43 ºF to 52 ºF (6℃ to 11℃), depending on the amount of added moisture (Kenny and Rollins, 2007). An adequate supply of high-quality steam is required to have an efficient pelleting operation (Reddy, 2011).

### *2.1.1.5 Conditioning time*

This is the amount of time that is required for heat and moisture to be exposed to the mash feed in the conditioner. Higher retention time result in greater degree of gelatinization to improve the pellet durability (Kenny and Rollins, 2007). Also, Bortone (2014) reported that, longer residence time in the conditioner allows more penetration of the moisture and better heat distribution, which leads to better binding of feed particles. This, therefore, increasing the pellet hardness and reduction of the fines produced.

#### *2.1.1.6 Die Selection*

Pellets are formed through roll pressing of the heated mash against metal die. Die can be varied in other to get the desired results on a particular formulation to be pelleted (Behnke, 2014). A die with a greater thickness (long die channel) has a positive impact on pellet quality. This is due to the higher flow resistance generated by a thicker die and the longer retention time under high pressure as the pellet passes through the die (Behnke, 2001). A thicker die has a larger L/d ratio and vice versa, and it represents the parameter in characterising a pellet die. Though thicker die will typically increase pellet durability but is negatively related to throughput (Production rate) and energy consumption (Fahrenhilz, 2012). Additionally, Lower L/d ratio can result in increased production, but pellet quality will likely suffer. However, it is necessary to have different dies of the same bore diameter but different effective thickness to optimize both pellet quality and production (Behnke, 2001).

#### *2.1.1.7 Drying and cooling*

Pellets are subjected to cooling to minimise the moisture content and temperature to levels that are safe for storage and allow easy handling. The relatively high-level temperature in the pellets is as a result of frictional heating of the die during the pelleting process (Adapa, 2013). Proper cooling is necessary to lower pellet temperature to about 8°C above the ambient temperature, and moisture content to be 12% (Mahmoud, 2017). Inadequate cooling and drying of pellets contribute to poor pellet quality, spoilage, heating, and spontaneous combustion, caking in storage bags and holding bins (Fasina, 1996; Biomass Energy, 2011). Rapid cooling result in the removal of more moisture and heat at the surface of the pellets than the core causing pellets to be brittle. However, prolonged cooling gives very dry pellets that can be subjected to abrasion and can be of low palatability (Mahmoud, 2017).

## 2.2 Different materials used as binders

### 2.2.1 Proteins

In feed manufacturing, especially the adhesive forces that proteins may exert are of interest. Protein can act as a binding agent between different feed particulates. Processing involves the combined effect of shear, heat, residence time and water resulting among others in partial denaturation of the protein in the feed. As has been shown by (Wood, 1987) partial denaturation during feed processing may positively affect the hardness and durability of the feed pellets.

Denaturation involves the breakdown of the (spatial) three-dimensional structure of the proteins (the secondary, tertiary and quaternary structures), thereby changing the bio-activity

of the protein (Van Barneveld *et al.*, 1993). Upon cooling, proteins reassociate, and so bonds can be established between the different particles.

According to Howell (1991) interactions that will affect the mechanical stability of proteins, and therefore may affect hardness and durability characteristics of pellet involve covalent bonding, electrostatic interactions, van der Waals-forces, hydrogen bonds and entropy factors. Conformational changes are the result of a change in the combination of all these forces. In some products like wheat, the presence and contribution of protein fibrils to pelletability can be the practical reason for differences observed in pellet quality (Moran, 1989). Fibrils formed by aggregated protein molecules are important for wheat dough structure and so for bread-making (Simmonds, 1972). Protein fibrils may act likewise in creating binding sites between particles in the pelleting process (Moran, 1989). Wood (1987) showed that there was an effect of the inclusion of raw or denatured protein on the physical quality of feed in terms of pellet hardness and pellet durability (Kahl and Holmen pellet criteria, respectively).

Processing may result in Maillard-reaction, in which many constituents of raw materials can participate, and that affects many quality attributes. Maillard reaction is a reaction between reducing sugars and free amino groups from amino acids, especially lysine (Voragen, 1995). Due to the formation of Maillard products, the animal utilization of protein and perhaps carbohydrates may be reduced. This phenomenon emphasizes the conflict between physical and nutritional quality of feed after processing (Goering, 1976; Van der Poel *et al.*, 1995).

### 2.2.2 Starches

Starch is one of the basic ingredients used in feed production. Chemically, starches from different sources exhibit different functional properties that need to be observed, not only in terms of nutrition but also from a technological aspect (Calmont, 2019). Starch is a biopolymer made of two types of macro-molecules; namely amylase and amylopectin (Brouillet-Fourmann *et al.*, 2003). Amylopectin composes of linear chains of glucose units linked together by α-1,4 glycosidic bonds and highly branched at α-1,6 positions by small glucose units at 10 nm intervals along the molecule's axis; it amounts between 70% to 85% of common starch (Durrani and Donald, 1995). Amylose and amylopectin combine to form granules through hydrogen bonding and arrange radially in layers. Starch granules range from 1 micron to 100 microns in diameter in a wide variety of sizes (Haralampou, 2000).

Starch may function amongst others as a binding agent or adhesive (Smith, 1983). Starches used for adhesive undergoes a heat or chemical treatment in which the properties of the native

starch are changed (Thomas *et al*., 1998). Smith (1983) stated that, the common way to affect functional properties of starches is gelatinizing of starch in the presence of water and heat, in the presence or absence of shear. Starch processing leads to crosslinking which may give the starch its desired properties as for instance changed rate and amount of swelling (Smith, 1983) which may be important for purposes in feed applications (Thomas *et al*., 1998).

When starch granules gelatinized, amylose suddenly forms double helices which aggregate (hydrogen-bonds) to each other and create semi-crystalline regions. Although, pellet binding occurs possibly by amylopectin as a result of the double helices formed at the non-reducing ends of the very large branched molecule which may aggregate with compatible starch or fibre surfaces on the different particles present during and after gelatinization (Schwartz and Zelinskie, 1978; Moran, 1989). According to Schwartz and Zelinskie (1978), the starch needs to be heated first in order to destroy its native structure and to allow reordering of the molecules which is needed to provide good binding properties in pellets.

## 2.3 Wheat

Wheat is a highly nutritional and widely cultivated cereal grain. It is one of the most popular crops in the world and holds the title of the world's second most developed grain, beaten only by maize (TGF, 2020). It provides 19% of calories and 19.8% of proteins in all food consumed (FAOSTAT, 2014; FAO-GCARD, 2012). In the 2017/18, more than 750 million tons of wheat were produced worldwide (TGF, 2020).

Wheat grain has three main parts, namely:

- The endosperm, or the protein/starchy part.
- The germ, the rich in protein/fat/vitamin part.
- The bran, the fibre-rich part.

**Wheat flour** is the powder obtained from grinding up parts of the wheat grain. There are different types of wheat flour, differentiated by the gluten content, their colour, and the grain parts used. Wheat flour is a key ingredient in bread, cakes, cookies, and most baked goods. The whole grain powder consists of all the three parts; the brown flour is made of the germ and bran, while white flour is produced from the endosperm only (Rattrey, 2019). Wheat flour is mostly starch (carbohydrate), but still contains some fibre and lots of protein. This protein is gluten, which is stretchy and makes flour great for baking.

**Wheat starch** is extracted from hydrated flour; upon a solvent treatment, the gluten matrix forms, and the starch is washed out. Wheat starch aids with texture, viscosity, gel formation, adhesion, binding, and moisture retention. It also works as an emulsifier, stabiliser and a clouding or glazing agent (Patel *et al.*, 2005).

Wheat starch thickens food through gelatinisation and retrogradation. Heat causes the starch to absorb water and swell and therefore increases viscosity and clarity. The strength of the gel depends on the type of starch being used and how much (Patel *et al.*, 2005). Since gluten (protein) is the sticky/gummy/stretchy part of flour, wheat starch does not have those glutinous properties. This is because the wheat starch processing removes gluten and the final product contains less than 20 ppm of gluten (Thompson, 2001).

**Wheat protein concentrate** is the doughy protein component extracted by wet processing of wheat flour. Its viscoelastic property makes it suitable for bakery products, noodles and other processed foods. The dough properties depend on the proper balance between glutenin (contributing to the strength and elasticity of dough) and gliadin (contributing to dough viscosity) (Uthayakumaran, 2017). It is the combination of these properties, which comprises the dough's functional properties (Wieser, 2007).

### 2.3.1 How to characterise starch in pelleting

Starch has a significant influence on pelleting. When starch is heated in the conditioner in the presence of water, it undergoes a transition phase called gelatinization. Starch begins to gelatinize when the granules are heated from 40°-120°C. The temperature required for gelatinization is dependent on the starch source and its amylose content (Haralampou, 2000). The gelatinisation occurs when water diffuses into the starch granule, which then swells significantly due to amorphous phase hydration causing loss of crystallinity and molecular order (Jenkins *et al.*, 1993; Jiménez *et al.*, 2012). Large starch granules create higher viscosity, but the viscosity is delicate because the granule's physical size makes it more sensitive to shear (Calmont, 2019). It can provide an extra binding capacity; thus, the narrow or large distribution of granule sizes has even more influence on gelatinisation.

Wheat starch, for example, has a bimodal distribution of both large and small granules, and those granules gelatinise at different moments in the conditioner depending on moisture available and heat. This enables smooth and easy control of the gelatinisation (Calmont, 2019).

## 2.4 Rheology of starch

Rheology, the study of flow and deformation of a material when an external force is applied. Immediately after gelatinization, starch paste forms and starch granules are increasingly susceptible to disintegration by shearing since they are swollen. Rheological properties describe the behaviour of materials subjected to shearing forces and deformation, which are considered viscoelastic complexes (Alcázar-Alay and Meireles, 2015). Other characteristics include texture, transparency or clarity, shear strength and the tendency for retrogradation. All these features play important roles in the commercial applications of starch (BeMiller & Whistler, 2009; Berski *et al.*, 2011). Rheological starch properties are studied through the behaviour of viscosity curves, which are controlled by concentration, temperature, and shear stress (Singh *et al.*, 2003).

The key factors that affect the rheological properties of starches are their source and the presence of other polymers (e.g. protein and hydrocolloid) (Sarker *et al*., 2013; Schirmer *et al.*, 2015). Many biopolymers that exist together with starch in aqueous mixtures interact in different ways to produce several features which influence the stability, texture, and quality of food products (Alcázar-Alay and Meireles, 2015). Rheology is widely acknowledged for its effect on the quality of feed and its sensory characteristics such as texture and appearance.

## 2.5 Feed particle binders

Pellet quality is related to feed processing equipment, condition, and feed formula. (www.bentoli.com, 2020) reported that the presence or absence of natural binders and inclusion of synthetic binders in feed are key formula variables that affect pellet quality. Binders are products used to bind, glue or hold the various feed ingredients together in order to maintain pellet integrity (Baudon and Hancock, 2003). Binders can be solids or liquids with the capacity of forming bridges, coatings or films that make strong inter-particle bonding (Paolucci et al., 2012).

### 2.5.1 Some useful application of feed binders

Inclusion of pellet binders in feed processing saves time, money and resources, and could enrich the stock quality by enhancing the quality of their feed (www.bentoli.com, 2020). Broiler chicken feed experiment with crumble feed containing high-quality pellet binders recorded a feed conversion ratio improvement of 4.8 percent when compared to crumble feed without a pellet binder (www.bentoli.com, 2020). Research by Lilly *et al*. (2011) shows that feed conversion ratios in broiler chickens will increase significantly when the amount or

percentage of fines are reduced in broiler feeds. A study by Lemons and Moritz (2016), Chicken feed performance improves significantly when fines in crumble feed are minimalized. With regards to aquaculture, feed and feeding represent almost half of the operational costs. An important factor in manufacturing aquatic animal feeds is the stability of the feed in water and its acceptability (Paolucci *et al.,* 2012). Tiamiyu and Solomon (2012), stated that binders are firming agents that are added to fish feed to improve the quality of pellets, water stability, hardiness, and bulk density. Hence, the inclusion of a binder is a necessity to ensure water stable feed with the purpose of increasing water stability with a concomitant decrease on nutrient loss (Sinha *et al.,* 2011) and this would have beneficial consequences on the aquaculture industry.

### 2.5.2 Different feed binders and the Market value

A number of products have been tested and a few have become widely used as pellet feed binders. These are broadly categorized based on their type into mineral binders (clays), Specialty binders (plant gums & starches), lignosulphonates (Lignin based binders), hemicellulose, CMC & other hydrocolloids, gelatin, molasses, wheat gluten & middling's, and others that include PMC and urea-formaldehyde. The market for pellet feed binder products has a remarkable impact on the animal meat industry. The size of the global feed binders' market is estimated in terms of value and Volume. A report published by marketsandmarkets.com, (2015) states that, in terms of value, the market for feed binders is estimated to grow at a CAGR of 3.4% to reach USD 4.96 billion from 2015 to 2020. As of 2014, the market was dominated by the Asia-Pacific region, where in China, India, and Indonesia were the fast-growing markets. The market in the Asia-Pacific is predicted to reach almost USD 1.63 Billion by 2020 and it is anticipated to remain a stronger market than European and North American regions (www.marketsandmarkets.com, 2015).

The feed binders' market has been earning more international presence and approval among customers. Competitors in the feed binders market include FMC Corporation (U.S.), Archer Daniels Midland Company (ADM) (U.S.), Darling Ingredients Inc. (U.S.), E. I. du Pont de Nemours and Company (U.S.), Borregaard ASA (Norway), and Roquette Freres (France). In 2016, Borregaard LignoTech introduced new pelleting aid and binder "Intact Aqua" for the aquafeed sector in Asia. It is expected that the Feed Binders market in the Asia Pacific region is to grow at a prominent rate. In Europe, Russia is deemed to witness remarkable growth in demand for feed binders, which can be the result of the reduction in meat import proposed by the government. In the Asia Pacific, countries such as Thailand and Malaysia, are predicted to

witness a high demand for feed binders, which can be credited to the growing demand for aquaculture activities (www.futuremarketinsight.com, 2017).

### 2.5.3 Guar gum

Guar gum is a gel-forming galactomannan obtained by grinding the endosperm portion of *Cyamopsis tetragonolobus* that belongs to the Fabaceae family (Mudgil *et al.,* 2014). The guar gum powder is a water-soluble polymer of β-1,4-D-mannose and β-1,4-D-galactose with some α-1,6 side chains (Storebakken, 1985). The large number of hydroxyl groups in guar gum increases its H-bonding ability when dissolved in water. This enhances the viscosity and gelling properties, making it useful as a thickener (Sharma *et al.,* 2018). A unique feature of guar gum is that it is high on galactose and mannose. Guar gum is used as a stabilising, thickening, suspending, and binding agent in foods and beverages. In pharmaceuticals and cosmetics, guar gum is used as a binding agent in tablets, and as a thickening agent in lotions and creams, respectively.

Among galactomannans, guar gum is easily available and the cheapest source. It is usually found in the Indian subcontinents, southern hemisphere in semi-arid zones of Brazil, South Africa, and Australia or the southern part of the USA, like Texas or Arizona. India and Pakistan produce a total of 90% of guar only out of which 80% is manufactured by India only (Poorna *et al.,* 2016).

#### *2.5.3.1 Hydrogen bonding activity of guar gum*

Hydrogen bonding activity of guar gum is due to the presence of hydroxyl group in guar gum molecule (Mudgil *et al.,* 2014). Guar gum shows hydrogen bonding with cellulosic material and hydrated minerals. With slight addition of guar gum, there is an alteration in electrokinetic properties of any system markedly (Schierbaum, 1971). Substitution of hydroxyl groups in guar gum with hydroxypropyl causes steric hindrance that decreases the stability of hydrogen bonds (Cheng *et al.,* 2002). Viscosity and hydration rate of guar gum does not remain constant but changes with conditions like temperature, pH, solute, concentration, etc. (Mudgil *et al.,* 2014).

### 2.5.4 LignoBond

LignoBond contains Calcium Lignosulphonate, a molecule that holds cellulose fibres together in the plant and is a very effective natural pelleting binder. It helps improve the hardness of pellets, feed blocks, cubes, and waters, therefore enhancing pellet durability, reduce dust and prevents segregation of the feed pellet. Since it is a soluble fibre of plant origin, it provides metabolizable energy and acts as a prebiotic (Omvik, 2012). LignoBond is the most cost-effective binder to improve pellet quality and press throughput in Europe, Asia and Brazil.

It is evaluated that there are more than 50 million tons of industrial lignin (lignosulfonate and alkali lignin) produced every year worldwide (Xiao et al., 2001), but only 10% of them are utilized, the rest being dumped as waste and making it an expensive item (Browning, 1975). According to Browning (1975), lignin and lignosulfonates are recognised feedstocks for the manufacture of low molecular weight aromatic chemicals. Their usefulness in the industry is mainly due to their colloidal properties. Statement (2011) stated that, lignosulfonate as an additive has been used over the years in the food or feed industry as a raw material in the production of vanillin, an emulsifier in animal feed and as a boiler water additive. The structures present in lignosulfonates are capable of strong adsorption at some solid-liquid interfaces by forming surface complexes with the solid (Catargiu, 2015). Lignosulfonate adsorption may be affected by either nonpolar Van der Waals' attraction, hydrogen bonding, ion exchange or covalent bonding (Browning, 1975).

### 2.5.5 Galactoglucomannas (GGMs)

Hemicelluloses are a primary component in lignocellulosic biomass, with galactoglucomannan (GGM) being the most abundant in softwood followed by arabinoglucuronoxylan. In nature, the second most abundant class of polysaccharides is hemicelluloses, and its advanced exploitation of is a fundamental for the development of sustainable wood-based biorefineries (Berglund *et al.,* 2019). Hemicelluloses structure is complex and varies widely among different types of plants. Galactoglucomannan consists of a backbone built up by D- mannose (Man) and D- glucose (Glc) units linked through β (1→ 4) glycosidic linkages. The mannose units can be branched with α (1→6) liked D-galactose (Gal), also O- acetylated at the C2 and C3 positions. In softwoods, galactglucomannan make up about 20% of the total mass, and xylan, the second most abundant hemicellulose, correspond to 5-10% (Sjöström, 1993). Galactoglucomannan is water soluble since it partly contains acetyl groups. According to

(Sihvonen et al., 1998; Hannuksela and Holmbom 2004), the affinity of galactoglucomannan to cellulosic fibres is strong thereby causing it to sterically stabilize colloidal wood pitch droplets. Galactoglucomannans are deacetylated and adsorb onto fibres during alkaline pulp treatment, resulting in decreased amount of dissolved GGMs available for stabilization of wood pitch droplets (Thornton *et al.,* 1991).

The cost for producing galactotoglucomannans is much higher than the cost of producing starch. Also, its commercial use is limited due to its monosaccharide composition and the degree and branching of its polymers (Lindqvist *et al.,* 2013). According to Willför *et al.,* (2003), hemicellulose has lured great interest not only because its extraction and purification can be carried out on a large scale, but also because wood-base polysaccharides do not contend with food production in contrast to starch which is a crucial point when using polysaccharides on an industrial scale.

## 2.6 Description of feed pellet quality

### 2.6.1 P-max (N/mm$^2$)

Pmax is a crucial parameter in pelletizing processes in terms of process energy consumption and pellet quality (Holm *et al.,* 2007, and Gilbert *et al.,* 2009). It directly shows the load level require to initiate pellet motion in the die and indirectly informs about the energy uptake of the pellet press (Mišljenović *et al.,* 2016). Pmax can be a good indicator to study the effects of pelleting materials on energy consumption. The difference in ingredient composition and water content have a strong influence on the pelletizing properties and thereby on the necessary pressure exerted by the roller (piston). This increase was attributed to the lack of water and low hemicellulose content in the torrefied spruce. Water molecules act as a plasticizer which increases the flexibility and softness of the material, resulting in lower friction within the press channel. On the other hand, hemicelluloses bind lignin and cellulose fibrils and provide flexibility in the plant cell wall (Jones *et al.,* 2003). The degradation of the hemicelluloses, cellulose and the lignin are likely to affect important pelletizing parameters such as the friction coefficient and Poisson ratio (thus, the measure of the effect, the phenomenon in which a material tends to expand in directions perpendicular to the direction of compression) which are directly correlated to pressure (Gilbert *et al.,* 2009). Also, (Stelte *et al.,* 2011) reported a clear correlation between particle size and pelletizing pressure (*P*x), indicating that friction increases

with decreasing particle size. This is because the smaller the particle size, the larger the surface area of contact between the pellet and the wall resulting in greater friction.

An increase in friction in the press channel of a pellet mill increases the pelletizing pressures which result in higher energy uptake of the mill and might decrease the capacity and in worst case an overheating (risk of fire) of a blockage of the mill's press channels (Stelte *et al.*, 2011)**.**

P-max can mathematics be express as:

P-max = $P_{N0}/U_{LR}$ ($e^{4uv}{}_{LR}{}^{C}$-1), C= x/2r where:

**$P_{N0}$** is a pre-stressing pressure incorporating plasticity in the model, **$\upsilon_{LR}$** is the Poisson's ratio. The first index **L** denoted the direction of applied stress (**L** = longitudinal fibre axes), and the second one R the direction of transverse deformation (**R** = radial fibre axes). **μ** is the friction coefficient and **c** is the compression ratio, defined as the ratio between the length of the pellet in the die (**x***)* and diameter *(***2r***)* (Stelte *et al.*, 2011)

### 2.6.2 Water activity (aw)

In commercial pet food, water activity has been an important parameter since the late 1960s (Carter and Fontana 2008). A major aspect of food quality is the stability of the final product. Water activity is one of the several important parameters that affect the stability of foods (Timmons, 2006). US FDA (2005) defines water activity as the quotient of the water vapor of a substance divided at the same temperature by the vapor pressure of pure water. Water activity measures the free or available moisture in foodstuffs and participates in and support physical, chemical reaction, biological reaction and spoilage processes. For feed stability, maximum water activity is critical. Scott (1957) demonstrated that microorganisms have a limiting rate of water activity level below which they will not grow. Below 0.6aw, spoilage by microorganisms would not be expected (Lowe and Kershaw, 1995). The water activity test is always done by Rotronic Hygrolab C1(Switzerland).

### 2.6.3 Hardness (N/mm)

The hardness refers to the weight (in kg) that the pellet can withstand without breaking. It has to be sufficient to withstand storage and transportation to the farm. The hardness of pellet depends upon the degree of expansion, raw materials used and processing parameters (Sørensen, 2012). It is an important indicator of feed quality. The better binding capacity of particles improves hardness. Tong (2017) reported that the hardness of a pellet is always represented by tensile strength and it defines the resistance of a pellet to break under a certain

tension, which is the main factor to evaluate the hardness. Tensile strength is among the most important parameters influencing pellet deformability and crushing (Claesson and Bohloli, 2002).

### 2.6.4 Moisture content (%)

Moisture is simply water diffused in a relatively small quantity. Nearly all materials contain at least a diminutive volume of moisture as a component of the molecular makeup. Moisture is given in the mass of materials; however, the relative proportion is dynamic and not constant.

Moisture content can be referring to as the amount of water in a substance or material. The water content of sample material is then referred to as moisture content in the testing and evaluation process of moisture analysis. Mahapatra *et al.*, (2010) stated the moisture content can affect the physico-chemical and stability of a pellets. Generally, Pellets exit the pellet mill at temperatures as high as 190°F and moisture contents are high as 17-18% (CPM, 2016). For proper handling and storage, pellets moisture content must be reduced to 10-12% and their temperature to about 15°F above atmospheric temperature (CPM, 2016).

# CHAPTER 3. MATERIALS AND METHODS

## 3.1 Background

The novel feed binders: Pa R5K, Pa R5K + Multifect (RNF), Pa R5K + Multifect (RNF) + CE17 and Pa R5K + Multifect (RNF) + NaOH were provided by KBM, Norwegian University of Life Sciences. They were galactoglucomannan with different acetylation levels prepared from Norwegian spruce by steam explosion. It was freeze-dried and stored in a cool, dry area. The wheat-based materials were obtained from the fôrTek storage room, Animal Science Department, NMBU. Realtek provided the single die pellet press and fôrTek know-how for single-die pelleting.

After the Steam explosion of the Norwegian spruce (*Picea abies*), the resulted galactomannans were named or labelled as:

1. Pa R5K (***P**icea **a**bies* **R**etentate from **5k**Da filtration), "(**B1**)"; which contains 83% GGM (58.9% mannose, 14.9% glucose, 9.4% galactose), 0.9% rhamnose, 2.7% arabinose and 13.7% xylose) and is the product recovered directly from steam explosion and ultrafiltration, from which other mannan samples were obtained from through enzymatic treatment and further purification.

Two kilograms of this base material were treated with a xylanase/xylosidase enzyme cocktail (Multifect) to breakdown the xylan based impurities into monosaccharides, followed by nanofiltration to remove the xylose from solution. Two kilograms of this sample were split into three equal batches for further treatment:

2. Pa R5K + Multifect (RNF), "(**B2**)"; which is considered as 98% pure GGM.
3. Pa R5K + Multifect (RNF) + CE17, "(**B3**)"; where after the Multifect treatment, the sample in solution was treated with *Ri*CE17 – a newly characterized mannan esterase active exclusively on the 2-*O*- acetylations of GGM (Michalak *et al.,* 2020), in order to reduce the degree of acetylation of the material prior to nanofiltration**.**

   Pa R5K + Multifect (RNF) + NaOH, "(**B4**)"; where the Multifect treated GGM was chemically deacetylated (completely) by adjusting the pH of the solution to 10 and overnight storage at room temperature.

DP- degree of polymerisation (chain length) = 3-15.

DA- degree of acetylation = 0.3 i.e. 30% mannose carries acetylations (this applies to 1 and 2).

### 3.2 Experimental design

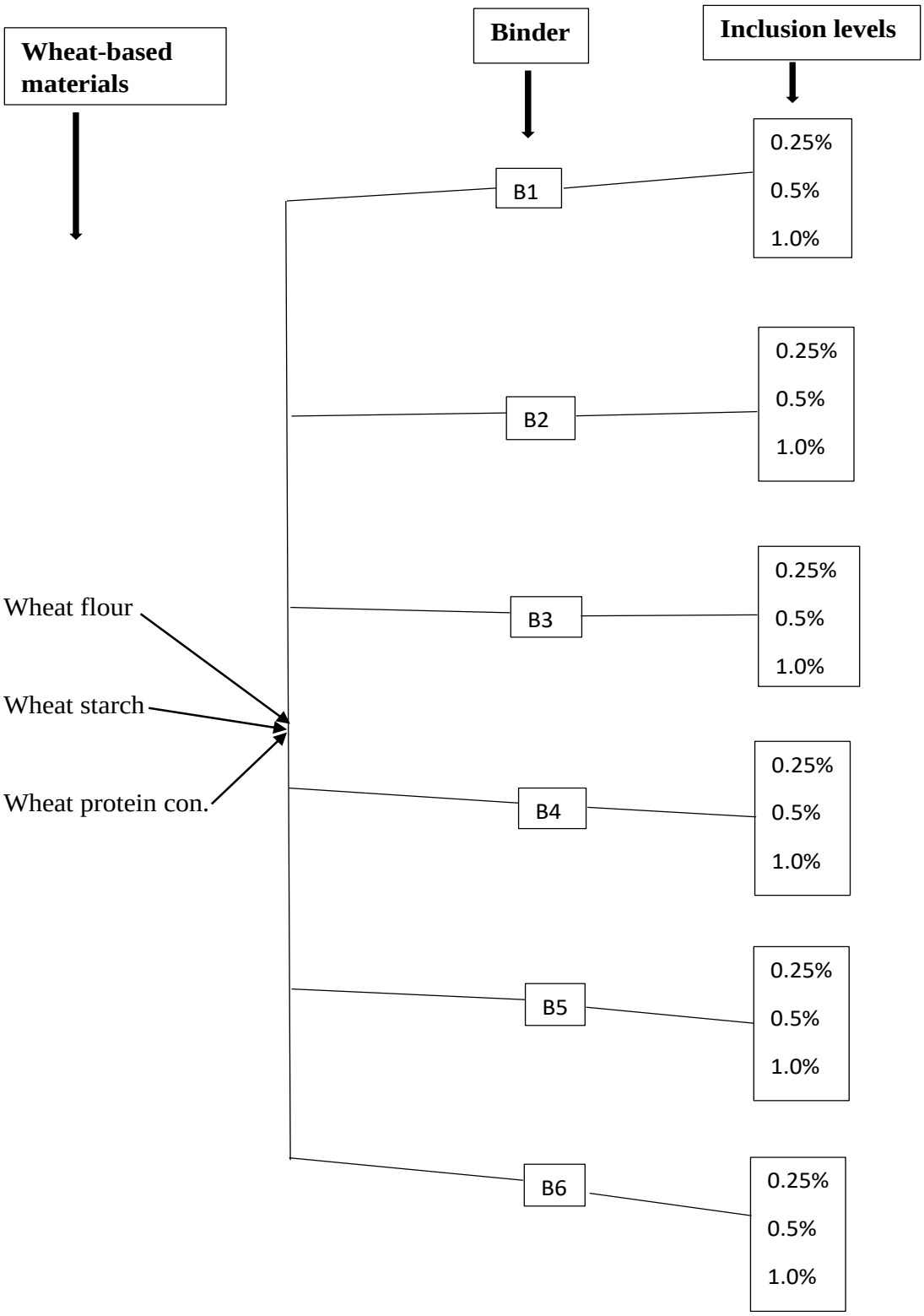



**Fig. 1** Flow chart of the experimental design

The experiment was made to consider a 1x6x3 multi-factorial design, where there is one of the three (3) wheat-based material, six (6) binders with three (3) inclusion levels. The three (3) wheat-based materials are Wheat flour, Wheat starch and Wheat protein concentrate and the six (6) different binders are four (4) non-commercial binders (Galactoglucomannans) and two (2) commercial binders (Guar gum and LignoBond) with inclusion rates (Dosages) of 0.25%, 0.5% and 1.0% (Fig. 1). Water was added once during mixing, to enhance gelatinisation, reduce friction during pelleting and result in decreasing the amount of fine generated and energy consumption (Table 1). Ten (10) replicated pellets or diet were made from each sample, and a total of 540 test pellets (diets) including positive and negative control pellets were made.

### 3.3 Milling of binders

The individual mannans were grounded to powdered form by a laboratory blender (Waring Commercial, 7010HB, Heavy Duty Lab Blender, USA) at the feed science Lab at the Animal Science department, NMBU. Grounding was done for 20 consecutive seconds and allowed 5 minutes for the dust particles to settle. Mortar and pestle were used for further grinding into fine powder since the blender were not able to give finer particles. Mechanical sieve shaker Retsch AS 200 Control at 150 amplitude, 1 minute and 0.2mm and 0.1mm Standardized Retsch sieves were used to sieve the samples to obtain the powdered samples and labelled them as; **B1, B2**, **B3** and **B4**. Guar gum and LignoBond were used as positive and negative control and labelled as **B5** and **B6,** respectively. The figures below are the materials used for milling and sieving the galactoglucomannans (binders).

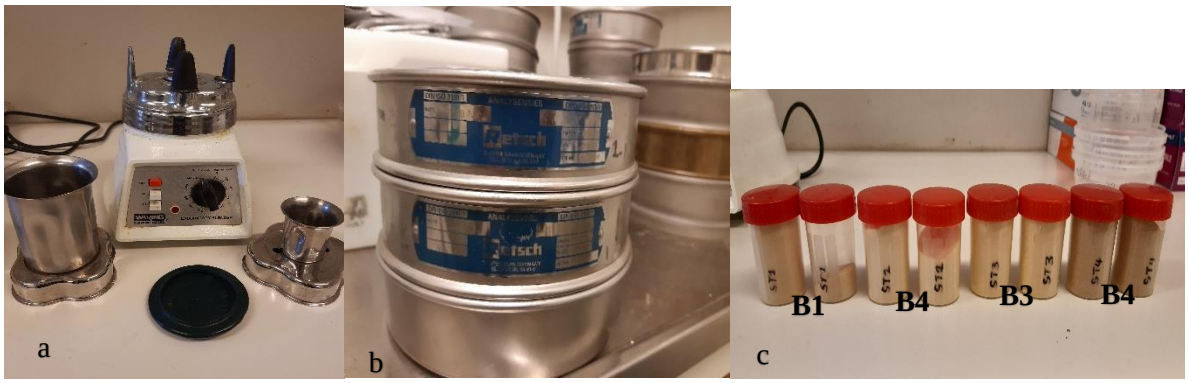



**Fig. 2**: Milling equipment: a= Blender, b= Sieve, c= Samples

## 3.4 Diet Formulation

**Table 1**. Details of the diet formulation

Each diet was made to contain 180g, and water was added to each diet depending on the initial water content of the wheat-based material.

| Ingredient | | B6 | | | B5 | | | B4 | | | B3 | | | B2 | | | B1 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.25 % | 0.5 % | 1.0 % | 0.25 % | 0.5 % | 1.0 % | 0.25 % | 0.5 % | 1.0 % | 0.25 % | 0.5 % | 1.0 % | 0.25 % | 0.5 % | 1.0 % | 0.25 % | 0.5 % | 1.0 % |
| Wheat-based (W.F, W.S, W.P.C) (g) | | 179.55 | 179.1 | 178.2 | 179.55 | 179.1 | 178.2 | 179.55 | 179.1 | 178.2 | 179.55 | 179.1 | 178.2 | 179.55 | 179.1 | 178.2 | 179.55 | 179.1 | 178.2 |
| Binders (g) | | 0.45 | 0.9 | 1.8 | 0.45 | 0.9 | 1.8 | 0.45 | 0.9 | 1.8 | 0.45 | 0.9 | 1.8 | 0.45 | 0.9 | 1.8 | 0.45 | 0.9 | 1.8 |
| Water addition (g) | W. F | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 | 8.7 |
| | W. S | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 | 9.48 |
| | W.P.C | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 | 14.16 |

W. F= Wheat flour, W. S= wheat starch, W.P.C= Wheat protein concentrate. Binders: PaR5K **(B1)**, PaR5K + Multifect (RNF) **(B2),** PaR5K + Multifect (RNF)+ CE17 **(B3),** PaR5K + Multifect (RNF)+ NaOH **(B4)**, Guar gum **(B5)** and LignoBond **(B6).** Inclusion levels: 0.25%, 0.5% and 1.0%.

### 3.5 Moisture measurement

The moisture content of wheat flour, Wheat starch, and Wheat protein concentrate was measured at Realtech Lab, using AXIS moisture analyser at Temperature difference (Td) of 160.0℃ (Fig. 3). Three (3) samples of each wheat-based material were measured and the average was recorded to represent the water content of the material (wheat-based). The average water content of the wheat flour was recorded 13%, wheat starch was 12.9% and wheat protein concentrate was 10%,


**Fig.3** AXIS moisture analyser

### 3.6 Mixing and water addition

To blend the wheat-based materials (wheat flour, wheat starch and wheat protein concentrate) and the binders, a premix was performed using a high shear mixer Diosna (Diosna P1/6, Germany) with a tulip-form chopper and three impellers mixer at a speed of 500 rpm and 250 rpm respectively for 3 minutes (shown in Fig. 4). A calculated amount of distilled water according to the diet formulation were sprayed using spraying lance (Düsen-Schlick GmbH, Germany, Model 970) assembled with Diosna mixer. An amount of 8.7g water were sprayed to samples with wheat flour, 14.2g of water were sprayed to samples with wheat protein concentrate and 9.5g of water were sprayed to samples with wheat starch. A homogeneous mixer of 16-18% moisture content was achieved for the individual samples. After addition

of water, the mixture was collected and packed into sealed plastic bags to prevent moisture loss. The samples were kept in a freezer for some weeks before the pelleting process started.

**Fig.4** Diosna P1/6, Germany

### 3.7 Cold Storage

The samples were stored in a -4℃ freezer and a fridge at 1℃ for more than 30 days before proceeding the conditioning and pelleting processes.

### 3.8 Heat Conditioning

An Eppendorf tubes with screw-cap was filled with 0.2g of each sample for each pellet. The tubes were tightly covered with a lid to prevent samples from being poured out when conditioning. They later placed in an Eppendorf tube rack and submerged into a boiling water bath (shown in Fig. 5) at boiling temperature and conditioned for 1 minute. The samples were taken out from the boiling water bath and allowed to cool for 15mins before pelleting. Cooling was required to avoid the samples being pelleted at different temperatures.

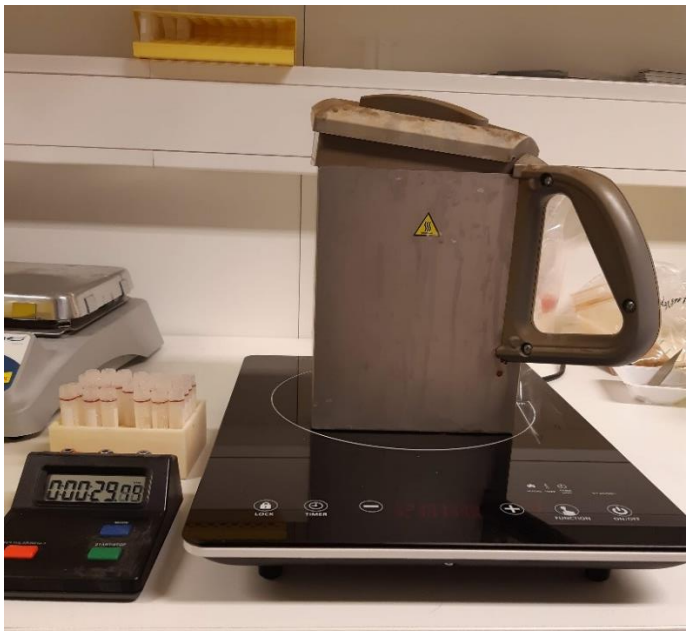


**Fig.5** Boiling or conditioning container

### 3.9 Pelletizing

The pellets used in this work were produced by a single die pellet press method described by (Salas-Bringas *et al.*, 2010 and Salas-Bringas *et al.,* 2011). The pelleting unit was assembled in a Lloyd LR 5K texture analyser (Lloyd instrument, U.K) (shown in Fig. 6), and it consists of a steel cylinder with a concentrically positioned compressing channel of 5.5mm in diameter in which a pressing rod with 5.4mm diameter was inserted to press the sample (diet) against a blank die. A jacket heater of 3000W, 230V and 16A is a temperature-controlled by PID (Proportional Integral- derivate) was used to heat the steel cylinder to a temperature of 81℃. This temperature level is required to eliminate Salmonella contamination in feed production (VKM, 2006).

The compression force applied was measured using 5KN load cell connected to a computer and data analysis software (Nexygen plus, version 4.0) using the maximal load force of 285Nm, which is to function as 12 bar pressure in the pellet press machine. When the temperature was set at 81℃, the die channel was filled with 0.2g of samples from the Eppendorf tube and the pressing rod was inserted into the die to avoid moisture escape during heating up. The samples were compressed at 2mm/min rate until a desired pelletizing pressure was reached. Afterwards, the pressure was released, and the blank die was removed.

The pellets were pushed or pressed out from the die by applying pressure on the pellet by the same arrangement used for palletisation at a compression rate of 10mm/min.

The compacted pellets were placed in sealed bags and stored in the fridge at the temperature of 4℃ for further analysis.

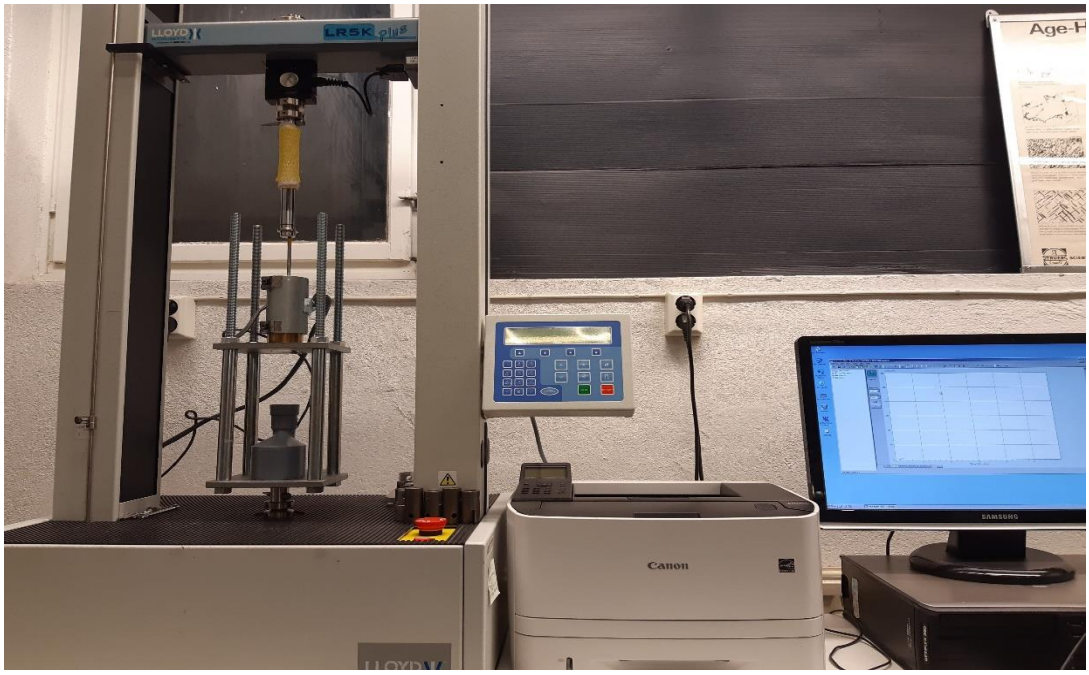



**Fig.6** Pelleting unit connected to Lloyd LR5K texture analyser and computer

## 3.10 Description of Laboratory Analysis

Laboratory analysis was done to obtain parameters such as water activity (aw), hardness (N/mm), moisture content and Surface roughness of the pellets. P-max values were determined during the production of the pellets.

### 3.10.1 Water activity (Aw)

The water activity (Aw) test was measured by a Rotronic Hygrolab C1(Switzerland) (shown in Fig. 7). The water activity values indicate the free water in pellets that could participate in physical, chemical, and biological reactions. Seven randomly selected pellets from each diet were tested twice and the average was recorded. The machine was set up to run the test until when it beeps to indicate the end of the test and the temperature was recorded.

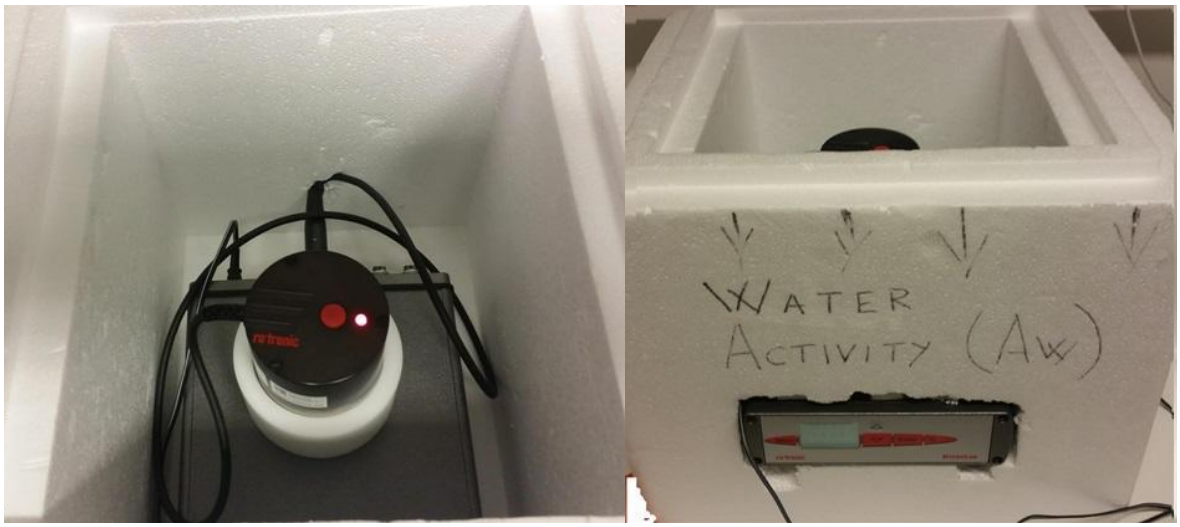



**Fig.7** water activity testing instrument, Rotronic Hygrolab

### 3.10.2 Tensile strength (Hardness) (N/mm)

Pellets strength was determined by using maximum peak force during the compression test. Before pellet breaking, the length and diameter of each pellet were measured with a digital calliper and recorded. The compression tests were performed using a flat surface probe of 60 mm in diameter (shown in Fig. 8) which was connected to the Lloyd LR 5K texture analyzer, by the same test arrangement used for the pelleting as described by Salas-Bringas *et al*. (2011). The compressing speed was set at 1 mm/min and the maximum normal force at pellet breakage was recorded.

The Brazilian test equation can calculate the Tensile strength:

($\sigma t$=2$P$/$\pi DL$=$P$/$\pi RL$)

Where:

$\sigma t$ is the tensile strength of pellet

P is the applied load or applied force to break the pellet

L is the thickness or length of the pellet

R is the radius; D is the diameter. Note: Diameter D = 2R.


**Fig.8** hardness test instrument (Lloyd LR5K texture analyser)

### 3.10.3 moisture content

The AXIS moisture analyser was used to measure the moisture content of the pellets. Mortar and pestle were used to break the pellet into smaller particles before moisture content was taken. Pellets was broken to increase the surface area and to expose available water in the pellet for measurement. The moisture analyser was run at the pre-set standard temperature and time (Td=160.0℃ and ts= 120sec).

## 3.11 Statistical Analysis

To study the effect of the six (6) different binders on the pellets (diet), the experimental data were analysed and presented as mean value with standard deviation. ANOVA was used to compare means at a significant level of 5% using Genstat software. P-values was used to test the effect of the binders on P-max ($N/mm^2$), water activity (aw), tensile strength (N/mm), and moisture content of pellet (%). Tukey-Kramer at a 95% confidence interval was used to test the significant difference among the treatments (binders). Pearson correlation at 95% confidence interval was used to analyse the correlation between tensile strength (N/mm) and water activity (aw).

# CHAPTER 4. RESULTS

## 4.1 P-max (N/mm$^2$)

The P-max (N/mm$^2$) represents the maximum peak flow force recorded for pelletizing the diets.

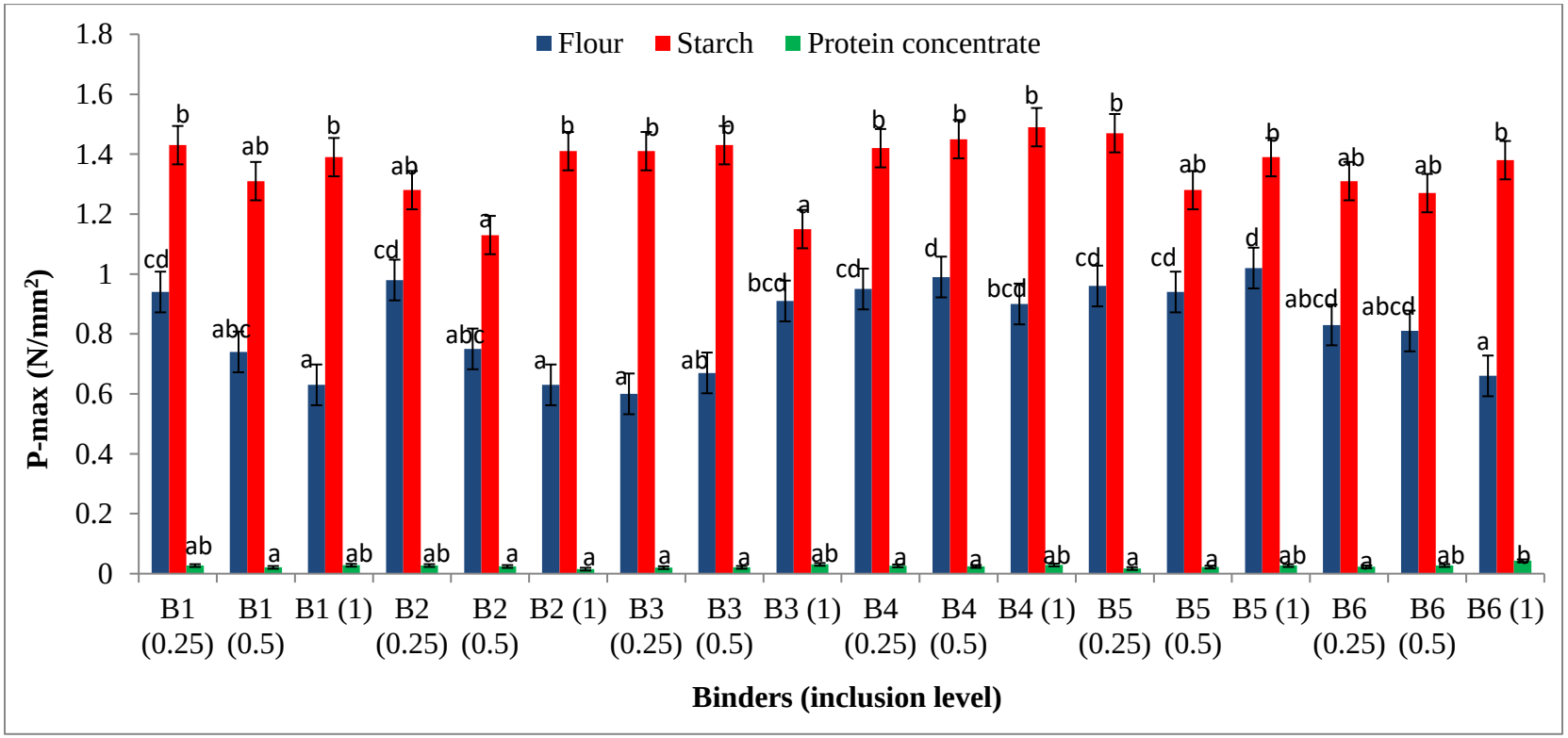



**Fig. 9**. P-max of the diets containing wheat flour, starch and protein concentrate with different binders at different inclusion levels. Different letters indicate significant differences for bars of the same colour; Error bars indicate standard error of means (SED). Test binders: PaR5K (B1), PaR5K+RNF (B2), PaR5K+RNF+CE17 (B3), PaR5K+RNF+NaOH (B4), control binders: guar gum (B5), and LignoBond (B6).

For wheat flour, comparing the test diet with the control diets, B4 at all inclusion levels had similar ($p>0.05$) P-max as both B5 (positive control) and B6 (Negative control). Except B6 level 1.0% where a significant difference ($P< 0.05$) was observed (Fig. 9). The test diets, B1 and B2 at inclusion levels 0.25% and 0.5% showed respectively, were not significantly different from B5 and B6. Though at least level 1.0%, B5 showed a significant difference among them.

The test diet B3 at level 0.25% and 0.5% were not significantly different ($P> 0.05$) from B6 at all levels. But, at level 1.0%, there was a significant difference ($P< 0.05$) between them. Again, no significant differences at level 1.0% was observed between B3 and B5. At the same time, B3 at 0.25% and 0.5% were significantly different from B5 at all levels (Fig. 9). Statistically, no significant effect ($P>0.05$) was observed between B1 and B2 at all levels. But B3 showed a significant difference from B1 and B2 at level 0.25% and 1.0%.

With the exceptions of B2 and B3 at levels 0.5 and 1.0 % respectively, no significant differences ($p>0.0$) in P-max for diet prepared from wheat starch were observed among all the binders and the inclusion level (Fig. 9).

Similar to the observations in diet prepared from wheat protein concentrate, no significant differences were observed among the binders and the inclusion levels except at B6 (Negative control) where the inclusion level 1.0% significantly ($p<0.05$) recorded a difference among others.

Generally, comparing pelleting the three wheat-based material and the binders, the results showed that, the P-max is highest at wheat starch, followed by wheat flour and wheat protein concentrates observed at the least (Fig. 9).

## 4.2 Water Activity (aw)

### 4.2.1 Water activity (aw) of wheat flour diets

**Table 2**. Water activity (aw) of the diets containing wheat flour with different binders at different inclusion levels. Results are presented as mean value ± SE.

| Binder | Inclusion level (%) | Water activity (aW) |
|---|---|---|
| B1 | 0.25 | $0.38^{def} \pm 0.003$ |
| | 0.5 | $0.37^{cde} \pm 0.001$ |
| | 1 | $0.36^{bc} \pm 0.003$ |
| B2 | 0.25 | $0.41^{g} \pm 0.002$ |
| | 0.5 | $0.38^{def} \pm 0.0007$ |
| | 1 | $0.41^{g} \pm 0.001$ |
| B3 | 0.25 | $0.37^{cde} \pm 0.002$ |
| | 0.5 | $0.37^{cde} \pm 0.001$ |
| | 1 | $0.39^{fg} \pm 0.0007$ |
| B4 | 0.25 | $0.43^{h} \pm 0.003$ |
| | 0.5 | $0.38^{def} \pm 0.003$ |
| | 1 | $0.41^{g} \pm 0.003$ |
| B5 | 0.25 | $0.43^{h} \pm 0.003$ |
| | 0.5 | $0.38^{def} \pm 0.002$ |
| | 1 | $0.41^{g} \pm 0.003$ |
| B6 | 0.25 | $0.33^{a} \pm 0.0007$ |
| | 0.5 | $0.34^{ab} \pm 0.001$ |
| | 1 | $0.37^{cd} \pm 0.01$ |
| F pr | | < 0.001 |

Means with same letter superscripts in vertical columns from the Tukey method indicates no significant different ($p>0.05$). Test binders: PaR5K (B1), PaR5K+RNF (B2), PaR5K+RNF+CE17 (B3), PaR5K+RNF+NaOH (B4), control binders: guar gum (B5), and LignoBond (B6).

The two-way ANOVA and Tukey's 95% confidence interval results showed that water activity was significantly ($p<0.05$) affected by the binders and the inclusion levels (Table 2).

The negative control, B6 had the least effect on water activity with no significant differences between levels 0.25 and 0.5%, but level 1.0% was significantly different. On the other hand, the positive control B5 had the highest effect on water activity where significant differences were observed among the inclusion levels.

Comparing the negative and positive control treatment (B6 and B5) with the test binders (B1, B2 and B3). The results showed no significant effects between the levels of the positive control B5 and the corresponding levels with test binder B4, although significant differences ($p<0.05$) were found among the levels within each binder (Table 2).

At the inclusion level of 0.25%, binders B4 and B5 significantly ($p<0.05$) recorded the highest water activity followed by B2 while B6 recorded the least (Table 2). No significant difference was observed between B1 and B3 at 0.25% and 0.5% levels. No significant differences ($p>0.05$) were observed among B1, B2, B3, B4 and B5 at inclusion level 0.5 %, but these were significantly ($p<0.05$) from that of B6. At the inclusion level of 1.0%, similar ($p>0.05$), water activity was observed between B1 and B6. Similarly, no significant difference was observed among B2, B3, B4 and B5 at 1.0%. However, between "B1, B6" and "B2, B3, B4 B5", there is a significant difference.

### 4.2.2 Water activity (aw) of wheat starch diets

**Table 3.** Water activity (aw) of the diets containing wheat starch with different binders at different inclusion levels. Results are presented as mean value ± SE.

| Binder | Inclusion level (%) | Water activity (aW) |
|---|---|---|
| B1 | 0.25 | $0.31^{c} \pm 0.003$ |
| | 0.5 | $0.29^{ab} \pm 0.001$ |
| | 1 | $0.30^{bc} \pm 0.0007$ |
| B2 | 0.25 | $0.35^{h} \pm 0.004$ |
| | 0.5 | $0.28^{a} \pm 0.001$ |

| | | |
|---|---|---|
| | 1 | $0.33^{fg} \pm 0.004$ |
| B3 | 0.25 | $0.30^{bc} \pm 0.001$ |
| | 0.5 | $0.33^{fg} \pm 0.003$ |
| | 1 | $0.34^{gh} \pm 0.011$ |
| B4 | 0.25 | $0.31^{c} \pm 0.0007$ |
| | 0.5 | $0.31^{c} \pm 0.004$ |
| | 1 | $0.33^{fg} \pm 0.004$ |
| B5 | 0.25 | $0.32^{def} \pm 0.0007$ |
| | 0.5 | $0.33^{fg} \pm 0.001$ |
| | 1 | $0.34^{gh} \pm 0.002$ |
| B6 | 0.25 | $0.33^{fg} \pm 0.006$ |
| | 0.5 | $0.31^{c} \pm 0.0007$ |
| | 1 | $0.33^{fg} \pm 0.0007$ |
| F pr | | < 0.001 |

Means with same letter superscripts in vertical columns from the Tukey method indicates no significant different ($p>0.05$). Test binders: PaR5K (B1), PaR5K+RNF (B2), PaR5K+RNF+CE17 (B3), PaR5K+RNF+NaOH (B4), control binders: guar gum (B5), and LignoBond (B6).

The two-way ANOVA analysis showed that the binders and the different inclusion levels affected water activity significantly ($p<0.05$). Binder B2 at inclusion levels 0.25% and 0.5 % significantly ($p<0.05$) had the highest and least effect on water activity, respectively (Table 3).

At the inclusion level of 1.0%, B2, B3, B4, B5 and B6 had similar ($p>0.05$) effect on water activity which were statistically different ($p>0.05$) from B1 at level 1.0 %.

At the inclusion level of 0.25%, B1, B3, and B4 had similar ($p>0.05$) effect on water activity. At the same level, no significant difference was observed between B5 and B6. However, water activity was statistically different ($p<0.05$) among “B1, B3, B4” “B2” and “B5, B6” at 0.25%.

Comparing to the control treatments (B5 and B6) to the test binders (B1, B2, B3 and B4), at least one level of the test binders had a similar effect on water activity as the controls (positive and negative).

### 4.2.3 Water activity (aw) of wheat Protein concentrate diets

**Table 4**. Water activity (aw) of the diets containing wheat protein concentrate with different binders at different inclusion levels. Results are presented as mean value ± SE.

| Binder | Inclusion level (%) | Water activity (aW) |
|---|---|---|
| B1 | 0.25 | $0.39^{abcde}$ ± 0.003 |
| | 0.5 | $0.38^{abcd}$ ± 0.002 |
| | 1 | $0.39^{abcde}$ ± 0.0007 |
| B2 | 0.25 | $0.40^{bcdef}$ ± 0.0007 |
| | 0.5 | $0.43^{fg}$ ± 0.003 |
| | 1 | $0.48^{h}$ ± 0.004 |
| B3 | 0.25 | $0.36^{a}$ ± 0.034 |
| | 0.5 | $0.36^{a}$ ± 0.0007 |
| | 1 | $0.35^{a}$ ± 0.001 |
| B4 | 0.25 | $0.40^{bcdef}$ ± 0.014 |
| | 0.5 | $0.40^{bcdef}$ ± 0.003 |
| | 1 | $0.35^{a}$ ± 0.006 |
| B5 | 0.25 | $0.42^{efg}$ ± 0.009 |
| | 0.5 | $0.38^{abc}$ ± 0.008 |
| | 1 | $0.41^{cdefg}$ ± 0.002 |
| B6 | 0.25 | $0.40^{bcdef}$ ± 0.008 |
| | 0.5 | $0.42^{efg}$ ± 0.003 |
| | 1 | $0.45^{gh}$ ± 0.015 |
| F pr | | < 0.001 |

Means with same letter superscripts in vertical columns from the Tukey method indicates no significant different ($p>0.05$). Test binders: PaR5K (B1), PaR5K+RNF (B2), PaR5K+RNF+CE17 (B3), PaR5K+RNF+NaOH (B4), control binders: guar gum (B5), and LignoBond (B6).

The two-way ANOVA and Tukey test showed statistically that significant effects on water activity ($p<0.05$) were observed among some of the pellets containing different binders.

In comparison, similar effects ($p>0.05$) on water activity were seen between the control binders (B5 and B6) and test binders B1, B2, and B4 at levels 0.25 and 0.5 %. On the contrary, significant differences ($p<0.05$) in water activity were observed at all inclusion levels between the control binders and B3, except B5 at 0.5% (Table 4). A significant difference ($p<0.05$) was seen among all levels of B2 and B3. Also, a significant effect was observed among B3 and B4 at level except B4 level 1.0%. The highest water activity was observed at B2 1.0% whiles the lowest were B3 and B4 at 1.0% (Table 4).

## 4.3Tensile Strength (N/mm)

### 4.3.1 Tensile strength (N/mm) of wheat flour diets

**Table 5.** Tensile strength (N/mm) of the diets containing wheat flour with different binders at different inclusion levels. Results are presented as mean value ± SE.

| Binder | Inclusion level (%) | Tensile strength (N/mm) |
|---|---|---|
| B1 | 0.25 | $9.94^{a} \pm 1.07$ |
| | 0.5 | $9.89^{a} \pm 2.53$ |
| | 1 | $8.38^{a} \pm 1.62$ |
| B2 | 0.25 | $8.14^{a} \pm 0.99$ |
| | 0.5 | $7.49^{a} \pm 0.88$ |
| | 1 | $7.55^{a} \pm 0.29$ |
| B3 | 0.25 | $7.82^{a} \pm 2.08$ |
| | 0.5 | $9.42^{a} \pm 1.31$ |
| | 1 | $10.33^{a} \pm 1.42$ |
| B4 | 0.25 | $8.76^{a} \pm 1.27$ |
| | 0.5 | $10.13^{a} \pm 1.14$ |
| | 1 | $7.61^{a} \pm 0.35$ |
| B5 | 0.25 | $7.51^{a} \pm 0.56$ |
| | 0.5 | $7.78^{a} \pm 0.70$ |
| | 1 | $7.67^{a} \pm 0.76$ |
| B6 | 0.25 | $6.58^{a} \pm 1.21$ |
| | 0.5 | $6.60^{a} \pm 1.56$ |
| | 1 | $7.93^{a} \pm 1.22$ |
| F pr | | 0.14 |

Means with same letter superscripts in vertical columns from the Tukey method indicates no significant different ($p>0.05$). Test binders: PaR5K (B1), PaR5K+RNF (B2), PaR5K+RNF+CE17 (B3), PaR5K+RNF+NaOH (B4), control binders: guar gum (B5), and LignoBond (B6).

The p-values of the maximum load from two-way ANOVA analysis showed that neither the binders nor the inclusion levels affected the tensile strength of wheat flour diet (Table 5). The results showed that B3 at level 1.0% recorded the highest tensile strength (10.33 N/mm) whiles B6 at level 0.25% recorded the lowest (6.58 N/mm). Yet, no significant differences($P>0.05$) was observed among B1, B2, B3, B4, B5 and B6 at all the inclusion levels.

### 4.3.2 Tensile strength (N/mm) of wheat starch diets

**Table 6.** Tensile strength (N/mm) of the diets containing wheat starch with different binders at different inclusion levels. Results are presented as mean value ± SE.

| Binder | Inclusion level (%) | Tensile strength (N/mm) |
|---|---|---|
| B1 | 0.25 | $3.85^{abcd} \pm 0.19$ |
| | 0.5 | $6.10^{e} \pm 0.50$ |
| | 1 | $4.31^{abcde} \pm 0.70$ |
| B2 | 0.25 | $2.85^{ab} \pm 0.77$ |
| | 0.5 | $2.47^{a} \pm 0.35$ |
| | 1 | $3.71^{abcd} \pm 1.13$ |
| B3 | 0.25 | $5.38^{cde} \pm 0.59$ |
| | 0.5 | $5.55^{de} \pm 0.13$ |
| | 1 | $3.39^{abc} \pm 0.10$ |
| B4 | 0.25 | $4.48^{abcde} \pm 0.26$ |
| | 0.5 | $4.63^{bcde} \pm 0.98$ |
| | 1 | $4.73^{bcde} \pm 0.62$ |
| B5 | 0.25 | $3.50^{abcd} \pm 0.99$ |
| | 0.5 | $4.75^{bcde} \pm 0.97$ |
| | 1 | $5.02^{cde} \pm 0.36$ |
| B6 | 0.25 | $4.36^{abcde} \pm 0.21$ |
| | 0.5 | $4.72^{bcde} \pm 0.10$ |
| | 1 | $3.85^{abcd} \pm 1.48$ |

| | |
|---|---|
| F pr | < 0.001 |

Means with same letter superscripts in vertical columns from the Tukey method indicates no significant different (p>0.05). Test binders: PaR5K (B1), PaR5K+RNF (B2), PaR5K+RNF+CE17 (B3), PaR5K+RNF+NaOH (B4), control binders: guar gum (B5), and LignoBond (B6).

The p-values of the maximum load from two-way ANOVA analysis showed that the effect on tensile strength was statistically not significantly (p>0.05) among binders B4, B5, B6 at all the inclusion levels. Also, B1, B2, and B3 at levels 0.25% and 1.0% showed no significant effect (p>0.05) on tensile strength compared to B4, B5 and B6. However, significant differences (p<0.05) were observed between "B1, B3" and "B2" at level 0.5% (Table 6). At B1level 0.5%, the value 6.10N/mm was observed as the highest tensile strength and 2.47N/mm at B2 level 0.5% recorded the lowest tensile strength. The lowest tensile strength observed showed no significant effect compared to B5 at level 0.25% and B6 at levels 0.25% and 1.0% (Table 6).

### 4.3.3 Tensile strength (N/mm) of wheat protein concentrate diets

**Table 7**. Tensile strength (N/mm) of the diets containing wheat protein concentrate with different binders at different inclusion levels. Results are presented as mean value ± SE.

| **Binder** | **Inclusion level (%)** | **Tensile strength (N/mm)** |
|---|---|---|
| B1 | 0.25 | $3.99^{bcd} \pm 0.55$ |
| | 0.5 | $1.91^{a} \pm 0.26$ |
| | 1 | $2.17^{ab} \pm 0.45$ |
| B2 | 0.25 | $4.05^{cd} \pm 0.41$ |
| | 0.5 | $2.25^{abc} \pm 0.67$ |
| | 1 | $2.39^{abcd} \pm 0.60$ |
| B3 | 0.25 | $2.84^{abcd} \pm 0.91$ |
| | 0.5 | $1.89^{a} \pm 0.14$ |

| | | |
|---|---|---|
| | 1 | $3.16^{abcd} \pm 0.61$ |
| B4 | 0.25 | $3.01^{abcd} \pm 0.76$ |
| | 0.5 | $2.98^{abcd} \pm 0.12$ |
| | 1 | $3.18^{abcd} \pm 0.21$ |
| B5 | 0.25 | $2.44^{abcd} \pm 0.25$ |
| | 0.5 | $3.28^{abcd} \pm 1.10$ |
| | 1 | $3.54^{abcd} \pm 0.37$ |
| B6 | 0.25 | $3.27^{abcd} \pm 0.89$ |
| | 0.5 | $4.01^{bcd} \pm 0.95$ |
| | 1 | $4.19^{d} \pm 0.27$ |
| F pr | | < 0.001 |

Means with same letter superscripts in vertical columns from the Tukey method indicates no significant different ($p>0.05$). Test binders: PaR5K (B1), PaR5K+RNF (B2), PaR5K+RNF+CE17 (B3), PaR5K+RNF+NaOH (B4), control binders: guar gum (B5), and LignoBond (B6).

The p-values from the two-way ANOVA analysis showed statistically that the different binders had no significant effect ($p>0.05$) on the hardness when compared to the positive and negative controls. Again, no significant differences were observed among the inclusion levels with any of the binders. However, the tensile strength recorded in B1 and B3 at level 0.5% were significantly different ($p<0.05$) from that of B1 and B2 at level 0.25 %, and B6 at levels 0.25 and 0.5% (Table 7). Statistically, no effect was seen between B1 and B3 at level 0.5% but they showed a significant effect among B6 at levels 0.5% and 1.0%. The highest tensile strength was observed at B6 level 1.0% and the lowest was at B3 level 0.5%.

## 4.4 Pearson correlations

The relationships between water activity and tensile strength in wheat flour, starch and protein concentrate are presented in Fig. 10, 11, and 12, respectively. A significant and positive relationship was observed between tensile strength and water activity in wheat flour (Fig. 10). However, there was a negative correlation between tensile strength and water activity in wheat starch which was not significant. This means, the water activity increases as the tensile strength decreases (Fig. 11). In the protein concentrate, non-significant positive relationship between tensile strength was observed (Fig. 12).

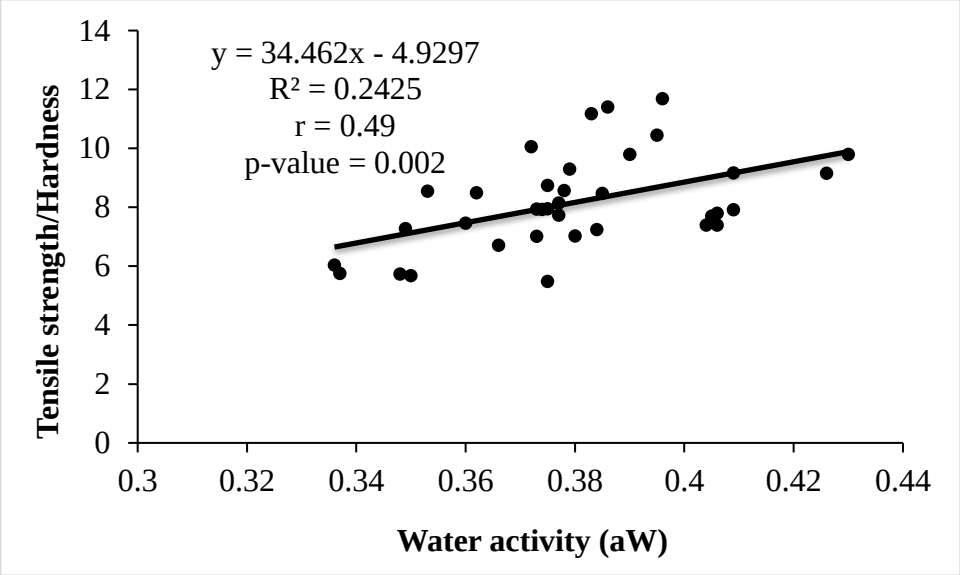



**Fig. 10**. Correlation between tensile strength (N/mm) and water activity (aw) of wheat flour. P-value represents whether the correlation coefficients are statistically significant or not, while $R^2$ shows the statistical measure of data being fitted to the regression line.

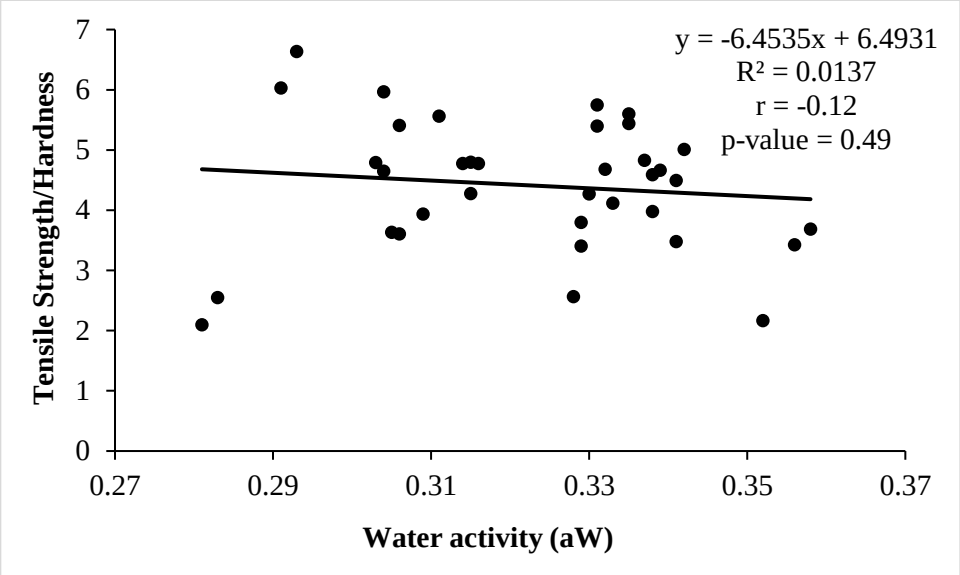



**Fig. 11.** Correlation between tensile strength(N/mm) and water activity (aw) of wheat starch. P-value represents whether the correlation coefficients are statistically significant or not, while $R^2$ shows the statistical measure of data being fitted to the regression line.

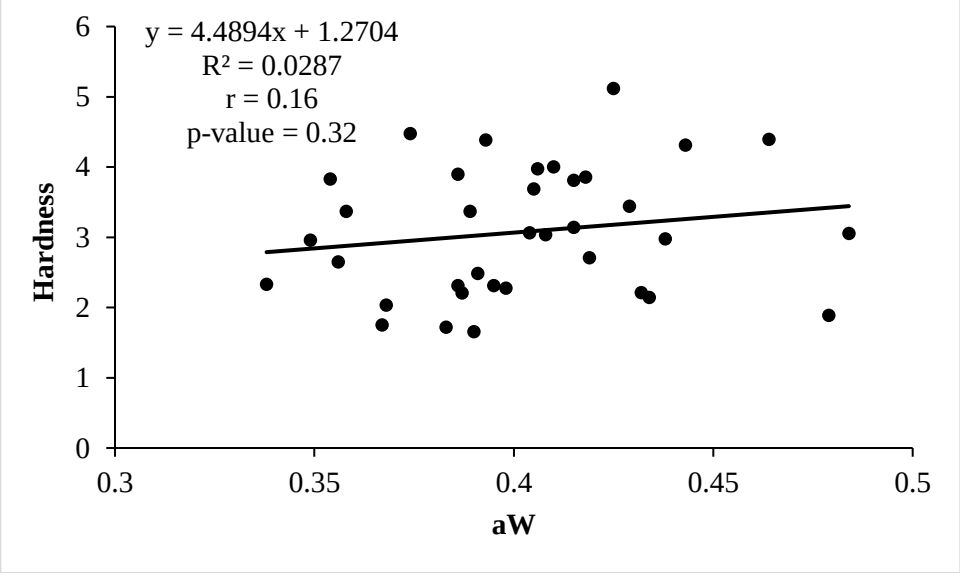



**Fig. 12.** Correlation between tensile strength (N/mm) and water activity (aw) of wheat Protein concentrate. P-value represents whether the correlation coefficients are statistically significant or not, while R2 shows the statistical measure of data being fitted to the regression line.

### 4.5.1 Moisture content (%) of wheat flour diets

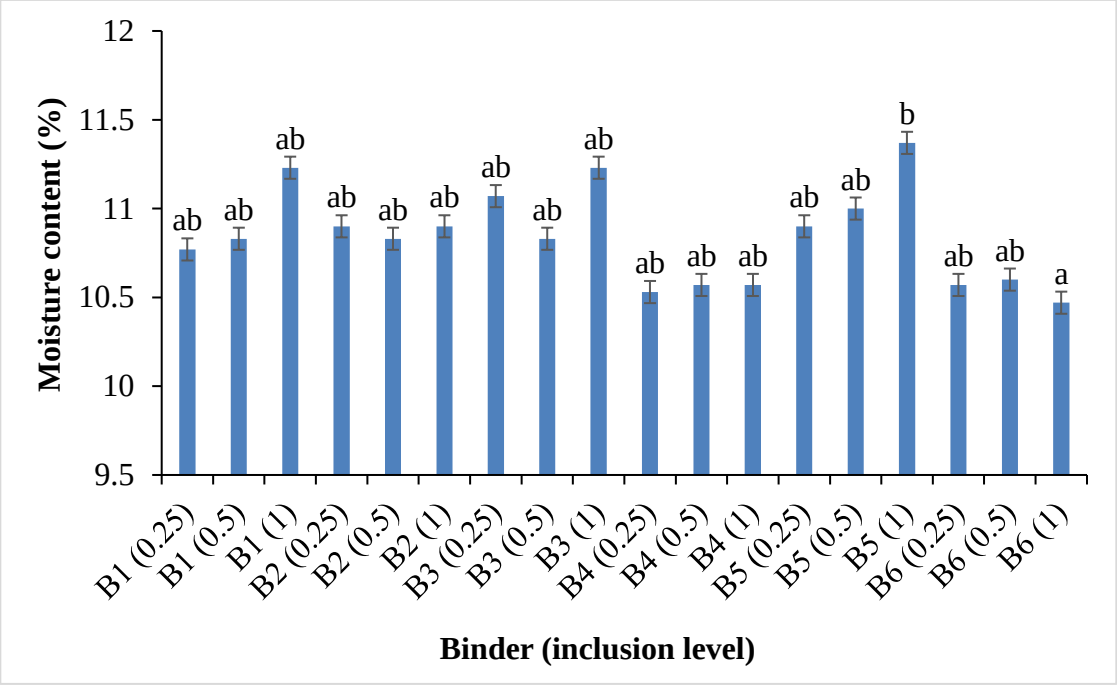



**Fig. 13.** Moisture content of the diets containing wheat flour with different binders at different inclusion levels. Different letters indicate significant differences at 0.05 probability level ($p<0.05$); Error bars indicate standard error of means (SED). Test binders: PaR5K (B1), PaR5K+RNF (B2), PaR5K+RNF+CE17 (B3), PaR5K+RNF+NaOH (B4), control binders: guar gum (B5), and LignoBond (B6).

The results in Fig. 13 showed that the binders have no significant effect ($P>0.05$) on moisture content after pelleting on the side of wheat flour, except at level 1.0% of B5 and B6 level where a significant difference ($P<0.05$) was observed.

### 4.5.2 moisture content (%) of wheat starch diets

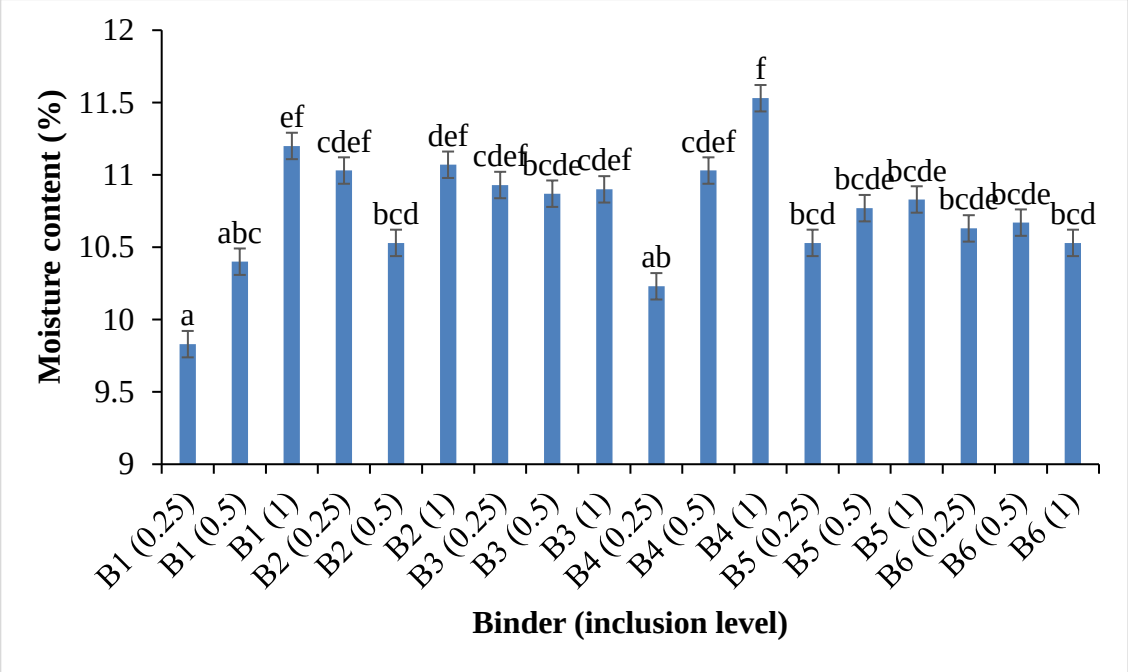



**Fig. 14**. Moisture content of the diets containing wheat starch with different binders at different inclusion levels. Different letters indicate significant differences at 0.05 probability level ($p<0.05$); Error bars indicate standard error of means (SED). Test binders: PaR5K (B1), PaR5K+RNF (B2), PaR5K+RNF+CE17 (B3), PaR5K+RNF+NaOH (B4), control binders: guar gum (B5), and LignoBond (B6).

The results in Fig. 14 showed that, there is no significant difference ($p>0.05$) between B5 and B6 at all levels on moisture content. Also, B2 and B3 at all levels showed no significant effect on the moisture content compared to B5 and B6. The highest moisture content was observed at B4 level 1.0%, and it was significantly different from B5 and B6, though B4 at level 0.25% and 0.5% had no effect. At B1 level 0.25%, the lowest moisture content was observed, and this is significantly different from B5 and B6 (Fig. 14).

### 4.5.3 moisture content (%) of wheat protein concentrate diets

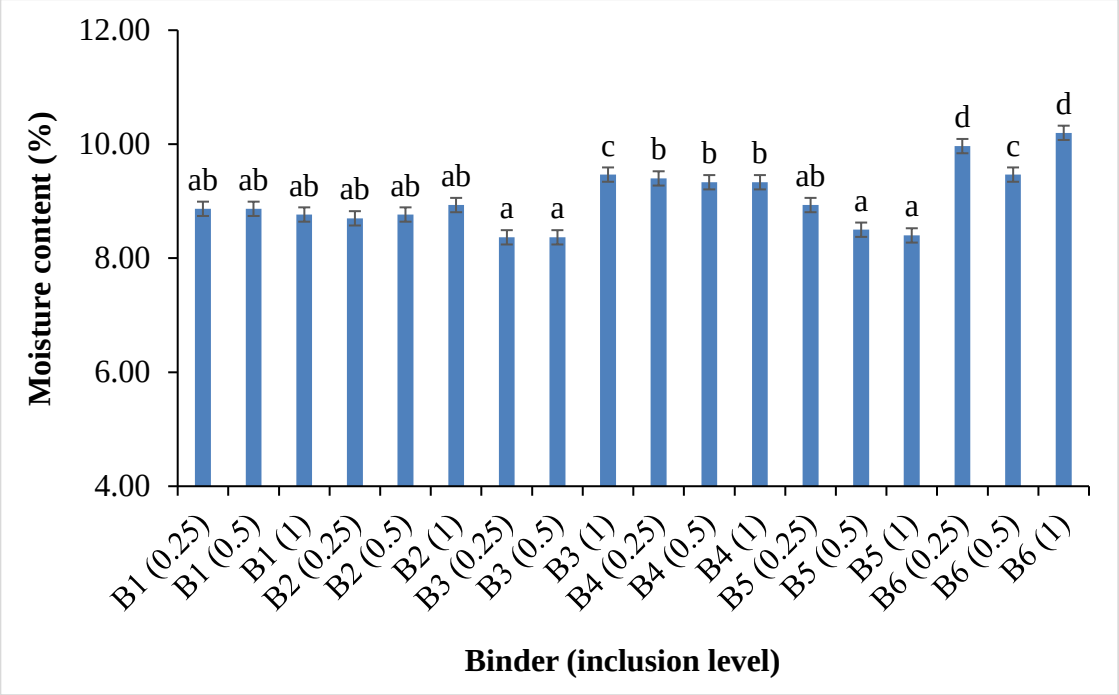



**Fig. 15**. Moisture content of the diets containing wheat protein concentrate with different binders at different inclusion levels. Different letters indicate significant differences at 0.05 probability level ($p<0.05$); Error bars indicate standard error of means (SED). Test binders: PaR5K (B1), PaR5K+RNF (B2), PaR5K+RNF+CE17 (B3), PaR5K+RNF+NaOH (B4), control binders: guar gum (B5), and LignoBond (B6).

The result based on wheat protein concentrate in Fig. 15 showed that there was no significant difference ($P>0.05$) among B1, B2, B3 and B 5 at all levels except B3 at level 1.0% where a significant difference ($P<0.05$) was observed. A significant effect was observed among the levels of B1, B2, B3 and B6, except at level 1.0% and 0.5% where B3 and B6 respectively showed no effect. Also, treatment B4 at all levels showed a significant difference ($P<0.05$) among both B5 and B6 at all levels except at B5 level 0.25% where no significant difference ($P>0.05$) was observed (Fig. 15).

# CHAPTER 5. DISCUSSION

## 5.1 Pmax (N/mm$^2$)

The pressure needed to push the compressed pellets out of the die channel was used as evidence of the friction generated between the pellet surface and the die channel area.

During pellet release, the pressure (P-max) were never overreached the load used to produce the pellets (i.e. 285N). The absolute values observed from pressure do not necessarily indicate the power requirements in large scale production. Yet, it can reveal the differences between materials and classify how difficult or easy a material would flow via a die.

From figure 9, the results based on wheat flour showed that Pmax values were higher at B4 level 0.5% and B5 level 1.0%. This indicates the high friction between die-pellet contact area. The higher friction might result from the treatment of acetylgalactoglucomannan with NaOH, which might have removed all the acetyl groups and decrease water solubility. Without acetyl groups, the water-solubility of acetylgalactoglucomannan is reduced due to the formation of inter- and intramolecular association (Willför *et al*., 2008).

On the other hand, at least one level of inclusion of each binder with wheat starch showed higher pelletizing pressure. This might be due to the high starch formulation influence over the treatment applied and causing higher friction across all pellets.

Comparing the energy consumption by the three wheat-based materials. The energy consumption of the pelletizing machine is less with wheat protein concentrate irrespective of the binders used. It could be the lack of starch in the wheat protein concentrate, which makes the protein material flow smoothly via the die due to less friction. According to FPL (1999), the coefficient of friction depends on the moisture content and the roughness of the surface, and it varies with ingredients. Lower friction in the die-pellet contact area will reduce the material retention in the pellet press, which will increase the material throughput or overall capacity of pelleting time.

## 5.2 Water Activity (Aw)

Water activity influences the role of microbial stability of ingredients and the final products. Every microorganism has a minimum water activity level at which growth is not possible because there is not enough water available to support pathogen growth (Fontana, 2000).

The pellets obtained from the experiment recorded water activity below 0.5aw which means microbial growth and reactivity are hindered. Again, chemical, and enzymatic activities are limited; therefore, pellet stability is maintained. The water activity level that limits the group of the vast majority of pathogenic bacteria is 0.90aw. The water activity level of 0.70aw is the lower limit for spoilage molds, while the limit for all microorganisms is 0.60aw (Rahman and Labuza, 1999; Carter and Fontana, 2008). Also, according to Timmons (2006), dry pet food and hard treats are in the 0.40 -0.50aw water activity range and at this low level of available water (<0.60aw) microbial stability is not an issue.

Based on wheat flour, B5 gave the highest water activity and B6 recorded the lowest (table 2). For wheat starch, the binders showed significant and non-significant difference among and within level of inclusions. But at least one of the levels of each of the test binders showed no significant difference with the control binders indicating its functional similarities with the control binders (table 3). For which protein concentrates, B3 gave the least water activity value followed by B1. The highest was recorded by B2 followed by B6, B5 and B4 (table 4).

The low water activity level recorded might be due to poor water-binding ability of the binders and vice versa. On the other hand, water activity level may result from high compacting pressure during the single pellet press, which eliminate free space for available water (Misljenovic *et al.*, 2016).

## 5.3 Tensile Strength/ Hardness of pellets (N/mm)

Tensile strength is an essential indicator of pellet qualities. It takes into account the amount of force to crush the pellets. It reflects pellet quality with respect to pellet strength and hardness, a pellet's resistance to breakage and dust generation during handling and transportation (Lu *et al.,* 2014). Sinka et al, (2007) observed in most pharmaceutical materials that the tensile strength increases as the compaction pressure are increased, and porosity decreases.

Based on wheat flour (Table 2), pellet with B6 (negative control) gave the lowest tensile strength, but B3 and B4 gave the higher tensile strength. This shows that B3 and B4 is more compact and higher strength is needed to break it. This may be due to molecular orientation and the formation of inter- and intra-molecular hydrogen bond because of the partial or complete removal of the acetyl group by CE17 and NaOH to decrease water solubility (Willför *et al.*, 2008).

Based on wheat starch (Table 3), a formulated pellet with B1 was more compact, followed by B3, therefore higher strength was required to break them. The B4, B5 (positive control) and B6 (negative control) also required a little less high strength to break compared to B1 and B3, showing no significant difference among them except at B1 level 0.5 %. The formulated pellet with B2 breaks at a lower strength.

For wheat protein concentrates (Table 4), pelleted feed with B6 was more compact, and more maximum forces were needed to break them compared to the others. The higher maximum force used to break B6 rather than others may be due to the strong interaction between lignosulphonate and protein, which creates better binding activity between their molecules. Lignin becomes soft under high temperature, which could help in particles bonding (Lu *et al.*, 2014). Also, protein plasticised under heat acts as a binder and has a positive effect on densified product (Winowiski, 1988; Briggs *et al.*,1999).

### 5.4 Correlation between hardness (N/mm) and Water activity (aw)

The positive correlation observed between the hardness and water activity is an agreement with Catargiu (2015) which stated that water activity affects the texture of the feed, thus lower water activity gives more hard, dry, and tough products.

### 5.5 Moisture content (%)

The moisture content after pelleting of all the diet with wheat flour seems to be same, and statistically, there is no significant difference ($P>0.05$) among them. A similar case was observed for wheat starch and wheat protein concentrate. Although for wheat protein concentrate, moisture content for B6 was higher than the others (Figure 13).

When comparing the three wheat-based materials, the diet from wheat starch and wheat have a moisture content between 9-12%, but the diets with wheat protein concentrate recorded the lowest moisture content.

This result is in accordance with CPM (2016), which stated that usually pellet exit the pellet press at temperature as high as 190$^{o}$ F with moisture contents as high as 17-18%. However, for proper storage and handling, pellets moisture content must be reduced to 10-12%. Also, Kraugerud (2008) indicated that pellets leaving the pellet mill require uniform dry of less than 13% moisture content to avoid mold or fungi. Since the binders did not have much influence on the moisture content of the pellet, the three wheat-based materials hold the account for the moisture content.

Nevertheless, these ingredients have differences in chemical compositions and physical states. Therefore, upon temperature and exposure time, they exhibit different abilities to lose or absorb moisture. Water is held by forces whose intensity ranges from the very weak forces retaining surface moisture to very strong chemical bond (Earle, 1966). Upon drying, it is evident that the water that is loosely held will be removed most easily. Also, the reduce moisture content may be caused by the evaporation due to the high temperature in the die hole (Misljenovic *et al.*, 2016).

# CHAPTER 6. CONCLUSION

The study assessed the use of galactoglucomannan and its derivates (B1, B2, B3, and B4) as a feed binder by using gaur gum (B5) and lignoBond (B6) as a reference binder.

The results according to the P-max, tensile strength, water activity and the moisture content after pelleting showed that, at least the test binders B1, B2, B3, and B4 have the same or similar effect as either B5 or B6. Thus, one inclusion level of either B1, B2, B3, or B4 and B5 or B6 showed no significant difference.

From the entire results, a close related effect was observed by B4 and B5. The same applies to B1, B2, and B6. But the impact of B3 was seen in all the binders.

The reference binders, guar gum (B5 positive control) and lignoBond (B6, negative control) are accepted and used commercially. Therefore, since the binding ability (results) of the test binders were evaluated to be between that of the control binders, then they could be used in place of the control binders to improve pellet quality. But for sustainable feed production, the availability, and the cost of the galactoglucomannan and its derivates must be considered and compared to the control binders. The cost of enzymes and further process for extracting B3 and B4 may increase production cost and reduce their use as binders.

# REFERENCE LIST


**Abdollahi**, M. R., Ravindran, V., Wester, T. J., Ravindran, G., & Thomas, D. V. (2013). The effect of manipulation of pellet size (diameter and length) on pellet quality and performance, apparent metabolisable energy and ileal nutrient digestibility in broilers fed maize-based diets. *Animal Production Science*, *53*(2), 114-120.

**Abdollahi**, M. R., Zaefarian, F., & Ravindran, V. (2019). Maximising the benefits of pelleting diets for modern broilers. *Animal Production Science*, *59*(11), 2023-2028.

**Adapa**, P. K., Tabil, L. G., & Schoenau, G. J. (2013). Factors affecting the quality of biomass pellet for biofuel and energy analysis of pelleting process. *International journal of agricultural and biological engineering*, *6*(2), 1-12.

**Alcázar-Alay**, S. C., and Meireles, M. A. A. (2015). Physicochemical properties, modifications, and applications of starches from different botanical sources. *Food Science and Technology*, *35*(2), 215-236.

**Alliance machinery (2012).** Binders in aanimal feed pelletin. http://www.pellet-machine.net/news/feed-pelleting-binders.html (2012).

**Amerah**, A. M., Ravindran, V., Lentle, R. G., & Thomas, D. G. (2007). Performance and digestive tract characteristics of broilers as influenced by particle size and feed form. In *Proceedings of the 19th Australian Poultry Science Symposium, Sydney, New South Wales, Australia, 12-14 February 2007* (pp. 85-88). Poultry Research Foundation.

**Baudon**, E. C & Hancock, J. D. (2003). Pellets diets for lactating sows. In Proceeding of Swine nutrition Ed. J. Hertramptf. Malaysia 5-7 th Nov. 2003.

**Behnke**, K. C. (1994). Factors affecting pellet quality. Maryland Nutrition Conference. Dept of Poultry Science and animal Science, collage of Agricultural, University of Maryland, collage Park.

**Behnke**, K. C. (2001). Factors influencing pellet quality. *Feed Tech*, *5*(4), 19-22.

**Behnke**, K. C., & Gilpin, A. (2014). Principles of mash conditioning. *Feed Pelleting Reference Guide. Kansas State University, Manhattan, Kansas, USA*.

**BeMiller**, J. N., & Whistler, R. L. (2009). Starch: chemistry and technology. Oxford: Academic Press.

**Berglund**, J., Azhar, S., Lawoko, M., Lindström, M., Vilaplana, F., Wohlert, J., & Henriksson, G. (2019). The structure of galactoglucomannan impacts the degradation under alkaline conditions. *Cellulose*, *26*(3), 2155-2175.

**Berski**, W., Ptaszek, A., Ptaszek, P., Ziobro, R., Kowalski, G., Grzesik, M., & Achremowicz, B. (2011). Pasting and rheological properties of oat starch and its derivatives. Carbohydrate Polymers, 83(2), 665-671. http://dx.doi.org/10.1016/j.carbpol.2010.08.036.

**Biomass Energy (2011)**. Biomass pellets production stages - part 2: pellet production. Available at: http://www.biomassenergy.gr/en/articles/technology/biomass/161-biomass-pellets-production-stages-part-2-pellet-production. Accessed on [2011-06-08].

**Bortone,** E., & Pas, D. (2014). Critical Steps in Mash Conditioning. *Feed Pelleting Reference Guide. Kansas State University, Manhattan, Kansas, USA*.

**Briggs**, J. L., Maier, D. E., Watkins, B. A., & Behnke, K. C. (1999). Effect of ingredients and processing parameters on pellet quality. *Poultry science*, *78*(10), 1464-1471.

**Brouillet-Fourmann,** S., Carrot, C., & Mignard, N. (2003). Gelatinization and gelation of corn starch followed by dynamic mechanical spectroscopy analysis. *Rheologica Acta*, *42*(1-2), 110-117.

**Browning,** W. C. (1975). The lignosulfonate challenges. Proceedings of the Eighth Cellulose Conference.I. Wood Chemicals - A Future Challenge.

**California Pellet Mill Co. (2016)**. The Pelleting Process. https://www.cpm.net/downloads/Animal Feed Pelleting. pdf (accessed 02-04-20).

**Calmont, M. (2019).** How the type of starch can influence pelleting. Borregaard LignoTech, Norway.

**Carter**, B., & Fontana, A. (2008). Water activity: The key to pet food quality and safety. *White Paper, Pet Food Int.*

**Catargiu,** A. M. (2015). Surface hydration and swelling rate of pellets in water made of microalgae with enzymes and lignosulfonates (Master's thesis, Norwegian University of Life Sciences, Ås).

**Cheng**, Y., Prud'Homme, R. K., Chik, J., & Rau, D. C. (2002). Measurement of forces between galactomannan polymer chains: effect of hydrogen bonding. *Macromolecules*, *35*(27), 10155-10161.

**Claesson**, J., & Bohloli, B. (2002). Brazilian test: stress field and tensile strength of anisotropic rocks using an analytical solution. *International Journal of Rock Mechanics and Mining Sciences*, *39*(8), 991-1004.

**Durrani**, C. M., & Donald, A. M. (1995). Physical characterisation of amylopectin gels. *Polymer gels and networks*, *3*(1), 1-27.

**Earle**, R. L. (2013). *Unit operations in food processing*. Elsevier.

**Fahrenholz,** A. C. (2012). *Evaluating factors affecting pellet durability and energy consumption in a pilot feed mill and comparing methods for evaluating pellet durability* (Doctoral dissertation, Kansas State University).

**Fahrenholz**, A. C. 2012. "Evaluating Factors Affecting Pellet Durability and Energy Consumption in a Pilot Feed Mill and Comparing Methods for Evaluating Pellet Durability." Ph.D. thesis, Kansas Universtiy, Manhattan, Kansas.

**FAO-GCARD** (2012). Breakout session P1.1 National Food Security. The Wheat Initiative, an International Research Initiative for Wheat Improvement, (www.wheatinitiative.org).

**FAOSTAT** (2014**)**. Wheat- the largest primary commodity. Crop and livestock systems, Crops and varieties for the improvement of food security. http://faostat3.fao.org,14/08/2014

**Fasina,** O. O. (1996). Cooling characteristics of alfalfa pellets.

**Fontana**, A. J. (2000,). Water activity's role in food safety and quality. In *Second NSF International Conference on Food Safety* (pp. 11-13).

**Forest Products Laboratory, FPL (1999).** Wood Handbook e wood as an Engineering material. General Technology Report FPL-GTR- 113, Forest Products Laboratory, Forest Service, U.S. Madison, WI: Department of Agriculture; 1999.

**Gao,** Q., Moechnig, B. W., & Crenshaw, J. D. (1999). Animal feed pelleting process and animal feed pellets produced therefrom. *U.S. Patent No. 5,871,802*. Washington, DC: U.S. Patent and Trademark Office.

**Gilbert**, P., Ryu, C., Sharifi, V., & Swithenbank, J. (2009). Effect of process parameters on pelletisation of herbaceous crops. *Fuel*, *88*(8), 1491-1497

**Goering**, H. K., (1976). A laboratory assessment on the frequency of overheating in commercial dehydrated alfalfa samples. *Anim. Sei*. 43 (4): 869-872.

**Hannuksela,** T., & Holmbom, B. (2004). Stabilization of wood-resin emulsions by dissolved galactoglucomannans and galactomannans. *Journal of pulp and paper science*, *30*(6), 159-164.

**Haralampou**, S. G. (2000). Resistant starch-a review of the physical properties and biological impact of RS3. Carbohydr. Polym. 41(3)285-292.

**Headly**, V. & Kershner. R. (1968). Adding animal fat to pelleted feeds. Feedstuffs 40(30).

**Holm,** J. K., Henriksen, U. B., Wand, K., Hustad, J. E., & Posselt, D. (2007). Experimental verification of novel pellet model using a single pelleter unit. *Energy & Fuels*, *21*(4), 2446-2449.

**Howell,** N. K. (1991). Protein-protein interactions. In: B.J.F. Hudson (Editor), Developments in Food Proteins 7. Elsevier Applied Science, 231 - 270.

**Jenkins**, P. J., Cameron, R. E., & Donald, A. M. (1993). A universal feature in the structure of starch granules from different botanical sources. *Starch-Stärke*, *45*(12), 417-420.

**Jiménez,** A., Fabra, M. J., Talens, P., & Chiralt, A. (2012). Edible and biodegradable starch films: a review. Food and Bioprocess Technology, 5(6), 2058 2076. http://dx.doi.org/10.1007/s11947-012-0835-4.

**Jones**, L., Milne, J. L., Ashford, D., & McQueen-Mason, S. J. (2003). Cell wall arabinan is essential for guard cell function. *Proceedings of the National Academy of Sciences*, *100*(20), 11783-11788.

**Kaankuka,** T. K., & Osu, D. T. (2013). Development of a revolving die and roller fish feed pelletizer. *International Journal of Engineering Innovations and Research*, *2*(1), 105.

**Kaliyan**, N., & Morey, R. V. (2009). Factors affecting strength and durability of densified biomass products. *Biomass and bioenergy*, *33*(3), 337-359.

**Kenny** M., & Rollin, D. (2007). Feed physical quality. Technical information for the broiler industry, Aviagen Technical Transfer Department. www.aviagen.com. 1007-AVN-004.

**Kraugerud,** O. F. (2008). Drying. Starch in feed, SEM. HFE 305 lecture note, NMBU. August 2019.

**Leaver,** R. H. (1988). The pelleting process. *Sprout-Bauer, Muncy, PA.*

**Lemons**, M. E., & Moritz, J. S. (2016). The effect of feeder space access and crumble-or pellet-to-fine ratio on 38-day-old broiler performance. *Journal of Applied Poultry Research*, *25*(1), 12-20.

**Lilly**, K. G. S., Gehring, C. K., Beaman, K. R., Turk, P. J., Sperow, M., & Moritz, J. S. (2011). Examining the relationships between pellet quality, broiler performance, and bird sex. *Journal of Applied Poultry Research*, *20*(2), 231-239.

**Lindqvist,** H., Holmback, J., Rosling, A., Salminen, K., Holmbom, B., Auer, M., & Sundberg, A. (2013). Galactoglucomannan derivatives and their application in papermaking. *BioResources*, *8*(1), 994-1010.

**Lopez,** J.G. (1993). Standardization of pellet water stability index techniques and the effects of cassava meal or vital wheat gluten on water stability and nutritional quality of pelleted diets for juvenile crayfish *orconectes naia*. PhD. Dissertation. Kansas State University, Manhattan.

**Lowe**, J. A., & Kershaw, S. J. (1995). Water activity-moisture content relationship as a predictive indicator for control of spoilage in commercial pet diet components. *Animal Feed Science and Technology*, *56*(3-4), 187-194.

**Lowe**, R. (2005). Judging pellet stability as part of pellet quality. *AFMA Matrix (South Africa)*.

**Lu**, D., Tabil, L. G., Wang, D., Wang, G., & Emami, S. (2014). Experimental trials to make wheat straw pellets with wood residue and binders. *Biomass and Bioenergy*, *69*, 287-296.

**MacBain, R. (1966).** Pelleting Animal Feed. Regional Feed School Presentation. American Feed Manufacturers Association. Arlington, VA., pp. 1-28.

**Mahapatra**, A. K., Harris, D. L., Durham, D. L., Lucas, S., Terrill, T. H., Kouakou, B., & Kannan, G. (2010). Effects of moisture change on the physical and thermal properties of sericea lespedeza pellets. *International Agricultural Engineering Journal*, *19*(3), 23-29.

**Mahmoud, F. (2017).** Good quality feed pellets- does it make sense? Feed planet magazine, zagazig university. https://en.engormix.com, 7/30/2019.

**Martin**, S. (1983). The effect of particle size on mixing and pelleting quality and production rates. In *First International Symposium on Particle Size Reduction in the Feed Industry, F-1 to F-14, Manhattan, KS.*

**McKee,** J.W. (1988). Effect of wheat gluten on underwater pellet stability of pelleted catfish and shrimp diets. M.S. Thesis. Kansas State University, Manhattan.

**Mišljenović,** N., Čolović, R., Vukmirović, Đ., Brlek, T., & Bringas, C. S. (2016). The effects of sugar beet molasses on wheat straw pelleting and pellet quality. A comparative study of pelleting by using a single pellet press and a pilot-scale pellet press. *Fuel processing technology*, *144*, 220-229.

**Moran Jr**, E. T. (1989). Effect of pellet quality on the performance of meat birds [Feed Manufacturers Conference, University of Nottingham].

**Mudgil,** D., Barak, S., & Khatkar, B. S. (2014). Guar gum: processing, properties and food applications—a review. *Journal of food science and technology*, *51*(3), 409-418.

**Muramatsu**, K., Massuquetto, A., Dahlke, F., & Maiorka, A. (2015). Factors that affect pellet quality: a review. *J. Agri. Sci. Tech. A*, *9*(2), 717-22.

**Omvik,** S. (2012). Nutritional impact of pellet binders. High performance natural pellet binder. LignoBond DD nature's best binder. Grain and Feed Milling Technology magazine, Jul/Aug 2012. http://www.lignotechfeed.com/Pelleting-Aids/LignoBond-DD[25/avr./2013 14:22:47].

**Paolucci**, M., Fabbrocini, A., Volpe, M. G., Varricchio, E., & Coccia, E. (2012). Development of biopolymers as binders for feed for farmed aquatic organisms. *Aquaculture*, *1*, 3-34.

**Patel,** B. K., Waniska, R. D., & Seetharaman, K. (2005). Impact of different baking processes on bread firmness and starch properties in breadcrumb. *Journal of Cereal Science*, *42*(2), 173-184.

**Pfost,** H.B., 1963. Testing the durability of pelleted feed, Feedstuffs. March 23: 66-68.

**Poorna**, K. S. V., Singh, A., Rathore, A., & Kumar, A. (2016). Novel cross-linked Guar gum -g- poly (acrylate) porous superabsorbent hydrogels: Characterization and swelling behaviour in different environments. *Carbohydrate Polymers*, 149, 175–185.

**Rahman**, M. S. & Labuza, T. P. (1999). Water activity and food preservation. *Handbook of food preservation*: 339-382.

**Rattray** D. (2019). What Is Wheat Flour? Buying, Baking, and Recipes. thespruceeat.com, 11/16/19.

**Reddy,** D.V. (2011). Feed pelleting technology including newer developments. In: Advanced Animal Nutrition. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp.291- 309.

**Reimer**, L. L., & Beggs, W. A. (1993). Making better pellets: Harnessing steam quality. *Feed Management*, *44*(1), 22.

**Richardson**, W., & Day, E.J., (1976). Effect of varying levels of added fat in broiler diets on pellet quality, Feedstuffs, 4800): 24.

**Salas-Bringas**, C., Filbakk, T., Skjevrak, G., Lekang, O. I., Høibø, O., & Schüller, R. B. (2010). Assessment of a new laboratory die pelleting rig attached to a texture analyzer to predict process-ability of wood pellets. Energy consumption and pellet strength. *Annual Transactions of the Nordic Rheology Society*, *18*, 77-86.

**Salas-Bringas**, C., Mišljenović, N., Lekang, O. I., & Schüller, R. B. (2011). Comparison between diametral and uniaxial compression tests of pelleted feed. *Annual Transactions of the Nordic Rheology Society*, *19*, 287-291.

**Salas-Bringas,** C., Mišljenovid, N., Wicklund, T., Lekang, O. I. & Schüller, R. B. (2011). Influence of particle size on strength of pelleted feed. *Annual Transactions of the Nordic Rheology Society*, 19: 293-301.

**Sarker**, M. Z. I., Elgadir, M. A., Ferdosh, S., Akanda, M. J. H., Aditiawati, P., & Noda, T. (2013). Rheological behavior of starch-based biopolymer mixtures in selected processed foods. *Starch-Stärke*, *65*(1-2), 73-81.

**Schierbaum**, F. (1971). Glicksman, M.: Gum Technology in the Food Industry, Academic Press, New York and London, 1969. XIII, 590 S., 8°, mit zahlreichen Abb. und Tab., Ganzleinen, Preis $27, 50. *Starch-Stärke*, *23*(10), 372-373.

**Schirmer,** M., Jekle, M., & Becker, T. (2015). Starch gelatinization and its complexity for analysis. Starch/Staerke, 67(1-2), 30-41. http://dx.doi.org/10.1002/star.201400071.

**Schwartz**, J. B., & Zelinskie, J. A. (1978). The binding and disintegrant properties of the corn starch fractions: amylose and amylopectin. *Drug Development and Industrial Pharmacy*, *4*(5), 463-483.

**Scott**, W. J. (1957). Water relations of food spoilage microorganisms. *Advances in food research*, *7*(1), 83-127.

**Sharma**, G., Sharma, S., Kumar, A., Ala'a, H., Naushad, M., Ghfar, A. A., & Stadler, F. J. (2018). Guar gum and its composites as potential materials for diverse applications: A review. *Carbohydrate polymers*, *199*, 534-545.

**Sievert**, D., & Pomeranz, Y. (1989). Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods. *Cereal Chemistry*, *66*(4), 342-347.

**Sihvonen,** A. L., Sundberg, K., Sundberg, A., & Holmhom, B. (1998). Stability and deposition tendency of colloidal wood resin. *Nordic Pulp & Paper Research Journal*, *13*(1), 64-67.

**Sihvonen,** A. L., Sundberg, K., Sundberg, A., & Holmhom, B. (1998). Stability and deposition tendency of colloidal wood resin. *Nordic Pulp & Paper Research Journal*, *13*(1), 64-67.

**Simmonds**, D. H. (1972). Wheat-grain morphology and its relationship to dough structure. *Cereal chemistry*.

**Singh**, N., Singh, J., Kaur, L., Singh, N., & Singh, B. (2003). Morphological, thermal and rheological properties of starches from different botanical sources. Food Chem. 2003, 81, 219–231.

**Sinha**, A. K., Kumar, V., Makkar, H. P., De Boeck, G., & Becker, K. (2011). Non-starch polysaccharides and their role in fish nutrition–A review. *Food Chemistry*, *127*(4), 1409-1426.

**Sinka,** I. C., Pitt, K. G., & Cocks, A. C. F. (2007). The strength of pharmaceutical tablets. *Handbook of Powder Technology*, *12*, 941-970.

**Sjöström**, E. (1993). Wood chemistry – Fundamentals and applications. London: Academic.

**Skoch**, E. R., Behnke, K. C., Deyoe, C. W., & Binder, S. F. (1981). The effect of steam-conditioning rate on the pelleting process. *Animal Feed Science and Technology*, *6*(1), 83-90.

**Skoch**, E.R., Binder, SF., Deyoe, C.W., Allee, G.L. & Behnke, KC. (1983). Effects of pelleting conditions on performance of pigs fed a corn-soybean meal diet, *J. Anim. Sci*., 57(4): 922-928.

**Smallman**, C. (1996). Maximising conditioning potential. *Feed milling international*, *190*(12), 30-1.

**Smith, P. S. (1983).** Food starches and their uses. In: D.L. Downing (Editor) Gum and Starch Technology Eighteenth Annual Symposium. *New York State Agricultural Experiment Station*, Geneva. 1 - 9.

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

**Statement**, E. (2011). Calcium lignosulphonate (40-65) as a food additive. *Food Standards*, Application A1030.

**Stelte,** W., Clemons, C., Holm, J. K., Sanadi, A. R., Ahrenfeldt, J., Shang, L., & Henriksen, U. B. (2011). Pelletizing properties of torrefied spruce. *biomass and bioenergy*, *35*(11), 4690-4698.

**Stelte,** W., Holm, J. K., Sanadi, A. R., Barsberg, S., Ahrenfeldt, J., & Henriksen, U. B. (2011). Fuel pellets from biomass: The importance of the pelletizing pressure and its dependency on the processing conditions. *Fuel*, *90*(11), 3285-3290.

**Stevens**, C.A. (1987). Starch gelatinization and the influence of particle size, steam pressure and die speed on the pelleting process. Ph.D. Thesis Kansas State University, USA, 86 pp.

**Storebakken**, T. (1985). Binders in fish feeds: I. Effect of alginate and guar gum on growth, digestibility, feed intake and passage through the gastrointestinal tract of rainbow trout. *Aquaculture*, *47*(1), 11-26.

**Subwilawan**, S., Tansakul, A., Sridokgmai, P., & Asavasanti, S. (2019). Effect of Moisture Adjustment on Physico-chemical Properties, Durability and Production Efficiency of Broiler Feed Pellets. *Applied Science and Engineering Progress*, *12*(2), 95-103.

**Supriya**, P., Rajni, B., & Rana, A. C. (2012). Pelletization techniques: A literature review. *International research journal of pharmacy*, *3*(3), 43-47.

**Swagelokenergy.com** (2009). Steam Quality – Plant Operations Require A High Steam Quality. Steam systems best practices. www.swagelokenergy.com, 888-615-3559.

**TFG** (2020). Grains, all you need to know about wheat. https://www.trafefinanceglobal.com.

**Thomas**, & Van der Poel. (1996). Physical quality of pelleted animal feed 1. Criteria for pellet quality. *Animal Feed Science and Technology*, *61*(1), 89-112.

**Thomas**, M., Van Vliet, T., & Van der Poel, A. F. B. (1998). Physical quality of pelleted animal feed 3. Contribution of feedstuff components. *Animal Feed Science and Technology*, *70*(1-2), 59-78.

**Thomas**, M., Van Zuilichem, D. J., & Van der Poel, A. F. B. (1997). Physical quality of pelleted animal feed. 2. Contribution of processes and its conditions. *Animal Feed Science and Technology*, *64*(2-4), 173-192.

**Thompson**, T. (2001). Wheat starch, gliadin, and the gluten-free diet. *Journal of the American Dietetic Association*, 101(12), 1456-1459.

**Thornton,** J., Eckerman, C., & Ekman, R. (1991). Effects of peroxide bleaching of spruce TMP on dissolved and colloidal organic substances. 6th ISWPC, Melbourne, Australia, April, *29*, 571-577.

**Tiamiyu**, L. O., & Solomon, S. G. (2012). Effects of different grain starches as feed binders for on-farm aqua-feeds. *Global Journal of Pure and Applied Sciences*, 18(1), 19-23.

**Timmons,** R. A. (2006). Water activity as a tool for predicting and controlling the stability of pet foods. Water activity for controlling the stability of pet foods – Engormix. *North American Biosciences Center, Alltech Inc.*, Nicholasville, Kentucky, USA.

**Tong**, A. (2017). Effect of different enzymes on compacting and physical quality characteristics of torula yeast *(Candida utilis)* (Master's thesis, Norwegian University of Life Sciences, Ås).

**US** Food and Drug Administration. (2005). Code of Federal Regulations, CFR 21, Part 110. Washington, DC.

**Uthayakumaran**, S., & Wrigley, C. (2017). Wheat: grain-quality characteristics and management of quality requirements. In *Cereal grains* (pp. 91-134). Woodhead Publishing.

**Van Barneveld**, R. J., Batterham, E. S., & Norton, B. W. (1993). Nutritional implications of heating proteins: the effect of heating protein concentrates on the digestibility and metabolism of lysine in growing pigs. *Recent Advances in Animal Nutrition in Australia*. Ed. Farrell, DJ, University of New England, Armidale, 201-212.

**van der Poel**, A.F.B., Verstegen, M.W.A., & Tarnminga, S. (1995). Chemical, physical and nutritional effects of feed processing technology. In: *16th Western Nutrition Conference*, 13-14 September 1995, Saskatoon Sask., Canada. 266 pp.

**VKM**. (2006). Assessment of the risk from Salmonella occurring in feeding stuffs and the feed production process (May 2006 ed., Vol. VKM Report 2006: 20): Norwegian Scientific Committee for Food Safety.

**Voragen,** A. G. J. (1995). Effects of some manufacturing technologies on chemical, physical and nutritional properties of feed. Recent advances in animal nutrition.

**Wieser**, H. (2007). Chemistry of gluten proteins. Food microbiology, *24*(2), 115-119.

**Willför**, S., Sjöholm, R., Laine, C., Roslund, M., Hemming, J., & Holmbom, B. (2003). Characterisation of water-soluble galactoglucomannans from Norway spruce wood and thermomechanical pulp. *Carbohydrate Polymers*, *52*(2), 175-187.

**Willför,** S., Sundberg, K., Tenkanen, M., & Holmbom, B. (2008). Spruce-derived mannans–A potential raw material for hydrocolloids and novel advanced natural materials. *Carbohydrate Polymers*, *72*(2), 197-210.

**Winowiski**, T. (1988). Wheat and pellet quality. Feed Management, *39*(9), 58-64.

**Winowiski**, T. S. (1995). Pellet quality in animal feeds (pp. 1-5). American Soybean Association.

**Wondra**, K. J., Hancock, J. D., Behnke, K. C., Hines, R. H., & Stark, C. R. (1995). Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. *Journal of Animal Science*, *73*(3), 757-763.

**Wood**, J. F. (1987). The functional properties of feed raw materials and their effect on the production and quality of feed pellets. Animal Feed Science and Technology, *18*(1), 1-17.

**www.bentoli (2020).** The benefits of pellet binders in broiler chicken feed. Bentoli.com.

**www.bentoli.com (2019).** The benefits of pellet binders in broiler chicken feed.

**www.futuremarketinsght.com (2017).** Feed Binder Market: Global Industry Analysis 2013 - 2017 and Opportunity Assessment; 2018 – 2028. Retrieved: 21.08.2019.

**www.marketandmarket.com (2015).** Feed Binders Market by Type (Clay, Plant Gums & Starches, Gelatin, Molasses, Lignosulphonates, Hemicellulose, CMC & Other Hydrocolloids, Wheat Gluten & Middlings, Others), Livestock (Poultry, Swine, Ruminants, Others), & by Region - Global Forecast to 2020. Feed binders' market, 2015, report code AGI 2304.

**Xiao**, B., Sun, X. F., & Sun, R. (2001). The chemical modification of lignins with succinic anhydride in aqueous systems. Polymer Degradation and Stability, *71*(2), 223-231.

**Yasothai,** R. (2018). Factors affecting pellet quality. International Journal of Science, Environment and Technology, *7*(4), 1361-1365.

# APPENDIX

## ANOVA TABLES

### Analysis of variance (Wheat flour)

Variate: aW

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Binder | 5 | 0.01464658 | 0.00292932 | 186.65 | <.001 |
| Inclusion_level | 2 | 0.00199800 | 0.00099900 | 63.65 | <.001 |
| Binder.Inclusion_level | 10 | 0.00387967 | 0.00038797 | 24.72 | <.001 |
| Residual | 18 | 0.00028250 | 0.00001569 | | |
| Total | 35 | 0.02080675 | | | |

### Analysis of variance (Wheat starch)

Variate: aW

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Binder | 5 | 0.00416581 | 0.00083316 | 63.41 | <.001 |
| Inclusion_level | 2 | 0.00317539 | 0.00158769 | 120.84 | <.001 |
| Binder.Inclusion_level | 10 | 0.00613328 | 0.00061333 | 46.68 | <.001 |
| Residual | 18 | 0.00023650 | 0.00001314 | | |
| Total | 35 | 0.01371097 | | | |

### Analysis of variance (Wheat protein concentrate)

Variate: aW

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Binder | 5 | 0.0254257 | 0.0050851 | 48.12 | <.001 |
| Inclusion_level | 2 | 0.0006422 | 0.0003211 | 3.04 | 0.073 |
| Binder.Inclusion_level | 10 | 0.0137932 | 0.0013793 | 13.05 | <.001 |
| Residual | 18 | 0.0019020 | 0.0001057 | | |
| Total | 35 | 0.0417630 | | | |

### Analysis of variance

Variate: P_Max_Flour

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 9 | 0.31460 | 0.03496 | 1.51 | |
| Rep.*Units* stratum | | | | | |
| Binder | 5 | 1.65836 | 0.33167 | 14.37 | <.001 |
| Inclusion_level | 2 | 0.22877 | 0.11438 | 4.95 | 0.008 |

| | | | | | |
|---|---|---|---|---|---|
| Binder.Inclusion_level | 10 | 1.66391 | 0.16639 | 7.21 | <.001 |
| Residual | 153 | 3.53223 | 0.02309 | | |
| | | | | | |
| Total | 179 | 7.39787 | | | |

## Analysis of variance

Variate: P_Max_Starch

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 9 | 0.18758 | 0.02084 | 1.00 | |
| Rep.*Units* stratum | | | | | |
| Binder | 5 | 0.59312 | 0.11862 | 5.69 | <.001 |
| Inclusion_level | 2 | 0.17478 | 0.08739 | 4.19 | 0.017 |
| Binder.Inclusion_level | 10 | 0.99993 | 0.09999 | 4.80 | <.001 |
| Residual | 153 | 3.18956 | 0.02085 | | |
| | | | | | |
| Total | 179 | 5.14496 | | | |

## Analysis of variance

Variate: P_Max_Protein_conc

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 9 | 0.0013867 | 0.0001541 | 1.41 | |
| Rep.*Units* stratum | | | | | |
| Binder | 5 | 0.0017044 | 0.0003409 | 3.12 | 0.010 |
| Inclusion_level | 2 | 0.0012478 | 0.0006239 | 5.70 | 0.004 |
| Binder.Inclusion_level | 10 | 0.0034256 | 0.0003426 | 3.13 | 0.001 |
| Residual | 153 | 0.0167333 | 0.0001094 | | |
| | | | | | |
| Total | 179 | 0.0244978 | | | |

## Analysis of variance

Variate: MC_Flour

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 2 | 1.48926 | 0.74463 | 8.80 | |
| Rep.*Units* stratum | | | | | |
| Binder | 5 | 2.55870 | 0.51174 | 6.05 | <.001 |
| Inclusion_level | 2 | 0.38037 | 0.19019 | 2.25 | 0.121 |
| Binder.Inclusion_level | 10 | 0.64630 | 0.06463 | 0.76 | 0.662 |
| Residual | 34 | 2.87741 | 0.08463 | | |
| | | | | | |
| Total | 53 | 7.95204 | | | |

## Analysis of variance

Variate: MC_Starch

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 2 | 0.11593 | 0.05796 | 1.29 | |
| Rep.*Units* stratum | | | | | |
| Binder | 5 | 1.50593 | 0.30119 | 6.72 | <.001 |
| Inclusion_level | 2 | 2.09926 | 1.04963 | 23.42 | <.001 |
| Binder.Inclusion_level | 10 | 4.02963 | 0.40296 | 8.99 | <.001 |
| Residual | 34 | 1.52407 | 0.04483 | | |
| Total | 53 | 9.27481 | | | |

## Analysis of variance

Variate: MC_Protein_conc

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| Rep stratum | 2 | 0.22259 | 0.11130 | 2.27 | |
| Rep.*Units* stratum | | | | | |
| Binder | 5 | 10.61648 | 2.12330 | 43.38 | <.001 |
| Inclusion_level | 2 | 0.81037 | 0.40519 | 8.28 | 0.001 |
| Binder.Inclusion_level | 10 | 3.04963 | 0.30496 | 6.23 | <.001 |
| Residual | 34 | 1.66407 | 0.04894 | | |
| Total | 53 | 16.36315 | | | |


**Norges miljø- og biovitenskapelige universitet**
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway