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Original research paper

RHEOLOGICAL PROPERTIES ASSESSMENT OF CARP FEED MIXTURES WITH DIFFERENT PROTEIN SOURCES USING MIXOLAB

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Abstract: Three carp formulations with same starch content (approximately 230 g/kg) with the inclusion of different protein sources were formulated. Control feed contained fish meal and soybean meal as a main source of protein which were completely substituted by dried duckweed. All ingredients were finely ground at the hammer mill and then mixed in twin-shaft paddle mixer. The aim of this paper was to assess the rheological and thermo-mechanical properties of these mixtures in order to forecast their behavior during subsequent processing. The determination of rheological properties was performed by Mixolab where water absorption capacity was set at a constant value of 100%. The Mixolab curve profiles varied where formulation in which the fishmeal was completely replaced by the novel plant protein ingredient, duckweed flour, displayed greatest resistance to deformation, therefore the highest water holding capacity which was in accordance with results obtained by the method used for assessment of water absorption properties. Obtained results demonstrated that Mixolab has a good potential to be used as a tool for screening dry feed mixes when taking into account the amount of water and steam necessary for the successful conditioning management. Further investigation is required to enhance the applicability of these methods in establishing more effective parameters during the conditioning process.

Keywords: *fish feed, Mixolab, starch rheology*

INTRODUCTION

The impact of processing conditions on pellet quality has been a significant area of focus, but testing ingredients and conditions often yields inconsistent results. However, changes in the physico-chemical properties of ingredient components have shown a more reliable link to pellet quality. It is demonstrated in some studies that variations in starch gelatinization correlated well with pellet hardness and durability (Thomas & Van der Poel, 2020). The main factor influencing the physical quality of pelletized feed is the variation in ingredient quality. This is followed by the particle size distribution, the conditioning process, die configuration, cooling methods, and drying techniques. Together, these elements are believed to contribute to 80% of the differences in physical quality (Cavalcanti & Behnke, 2005).

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The Mixolab device is commonly employed for rheological assessment of flours to predict dough behavior throughout baking processes, including mixing, cooking, and cooling. By measuring torque during mixing under controlled temperature conditions, it enables the determination of key factors such as the water required for dough development, the duration of dough development, protein strength and breakdown, starch gelatinization, hot gel stability, enzymatic activity, and starch retrogradation during the cooling phase (Bressiani, 2019). Extrusion processing is a commonly used method in fish feed production. During this process, a mixture of ingredients is transformed into a melt under the combined effects of high temperature, pressure, and shear forces (Sørensen, 2012). During extrusion cooking, several reactions occur, including gelatinization, protein denaturation, shearing, mixing, and hydration. The changes in starch granules play a crucial role in predicting the behavior of the resulting paste and its impact on subsequent processing steps. Throughout the extrusion process, up to 90% of the starch is gelatinized, leading to the intertwining of the ingredients (Kannadhason Muthukumarappan & Rosentrater, 2011). In addition to increased temperature and water content, starch conversion is also affected by other factors, such as proteins and fats (Lin, Hsieh & Huff, 1997). The internal lipids within native cereal starches influence the swelling and gelatinization behavior of the starch granules. Additionally, amylose-lipid complexes within the starch granules significantly limit water penetration into these complexes. This restriction occurs because the amylose fraction of the starches has the capacity to bind to lipid compounds, such as fatty acids (Abd El-Khalek & Janssens, 2010). Varying levels of protein inclusion in mixtures were observed to affect network structure during gelatinization. Consequently, starch interactions with various ingredients play a crucial role in feed extrusion. Moisture is essential for both the gelatinization of starch and the hydration of proteins (Sørensen, 2012). Excess moisture in the feed is necessary for complete starch gelatinization when combined with elevated temperature. If there is limited amount of water, starch gelatinization occur at higher temperatures and more time is needed in order for gelatinization to be complete (Abd El-Khalek and Janssens, 2010). Before the extrusion process in fish feed production, the mixture undergoes preconditioning phase in which the dry material is combined with water and steam in order for it to reach the required moisture content and temperature. This step enhances heat transfer during the friction process in the extruder, reduces wear, and decreases energy consumption (Sørensen, 2012). Conditioning is a crucial step in producing high quality pellets. The amount of steam and heat that is necessary for successful conditioning mainly depends on the properties and inclusion levels of different ingredients (Thomas &Van der Poel, 2020). The objective of this study was to evaluate the thermo-mechanical and water absorption properties of three fish feed mixtures with identical starch content and particle size, but differing protein sources. Analysis of water absorption properties is an easily performed method that can potentially be used in fast evaluation of feed mixes which would enable us to establish more effective parameters during the conditioning process. Understanding the functional properties of mixes and their interrelationships can assist us in more effectively planning critical steps in fish feed production. This, in turn, will help achieve a more desirable outcome in terms of process efficiency and the quality of the final product.

MATERIALS AND METHODS

Materials

The experimental diets were formulated using the following ingredients: wheat, sunflower meal, soybean meal, duckweed (*Lemna minor*), fishmeal, poultry meal, rapeseed oil, and calcium carbonate ($CaCO_3$). Source of each used ingredient is given in details in Table 1.

Fish feed formulation and preparation

Three carp feeds (control, formulation 1 and formulation 2) with same starch content (approximately 230 g/kg) and with inclusion of different protein sources were formulated as presented in Table 1. Control formulation was created to have approximately 340 g/kg of protein and 70 g/kg of fat. In the other two formulations conventional protein sources of fish feed, fishmeal (Formulation 1, F1) and soybean meal (Formulation 2, F2), were replaced with novel plant protein ingredient – duckweed powder. For each mix dry ingredients were ground by a hammer mill (ABC Inženjering, Pančevo, Serbia) equipped

Table 1.
Ingredient and chemical composition of control and two experimental formulations

Ingredients (g/kg)	Control	F1	F2
Wheat[a]	320	320	320
Sunflower meal[b]	235	235	235
Soybean meal[c]	100	100	0
Duckweed[d]	0	200	100
Fishmeal[e]	200	0	200
Poultry meal[f]	80	80	80
Rapeseed oil[c]	35	35	35
$CaCO_3$[a]	30	30	30
Chemical composition (g/kg)			
Crude protein	337.70	275.60	366.40
Crude fat	67.90	58.30	73.70
Crude ash	95.10	77.70	94.20
Starch	229.50	229.99	229.10

Supplied from a local feed mill, Serbia; [b] Victoriaoil, Šid, Serbia; [c] Sojaprotein, Bečej, Serbia; [d] McSelect, Parabel USA Inc., Fellsmere, Florida, United States; [e] Hanstholm NSM, Hanstholm, Denmark; [f] SONAC Poultry meal 65 Premium, Sonac Usnice SP.z.o.o., Usnice, Poland

with the 1.0 mm sieve and then mixed in a double-shaft paddle mixer (model SLHSJ0.2A Muyang, Yangzhou, China). Oil was added via six spray nozzles which were placed above paddles.

Proximate composition

The proximate composition of the formulations (Table 1) was determined by the laboratory FINSLab, analytical provider of the Institute of Food Technology, University of Novi Sad. The formulations were analyzed for moisture content (AOAC Method 934.01), crude ash content (AOAC Method 942.05), crude protein content (AOAC Method 978.04), crude fat content (AOAC Method 920.39) and starch content (AOAC Method 996.11).

Mixolab measurements

Mixolab™ device (ChopinTechnologies, France) was used to study the thermo-mechanical behavior of the mixtures. The Chopin+ protocol with the modification of dough mass to 90 g was employed. The following set-up was used in the experiment: constant mixing speed of 80 rpm, hydration % (14 % base) with a constant hydration of 100%. High water absorption values were selected in order to allow accurate measurement of samples that absorb larger amounts of water. Taking into account the composition of our mixtures, which included different sources of protein and fiber (e.g. sunflower meal and duckweed), which can significantly influence the absorption capacity, hydration level of 100% was targeted to ensure uniformity of the mixtures and to prevent the formation of non-homogeneous agglomerates. This assumption was confirmed by Lovegrove et al. (2020), who observed that the addition of fiber components improved water absorption, and Álvarez-Castillo, Aguilar, Bengoechea, López-Castejón and Guerrero (2021), who reported that the addition of alginate in soy protein formulations led to an enhanced water uptake capacity. Otherwise, these samples would not form dough, and the measurements could not be performed. Temperature regime was set at 30 °C for the first 8 min and then heated to 90 °C over 15 min at the rate of 4 °C/min. Samples were held at 90 °C for 7 min and then cooled to 50 °C over 10 min at the rate of 4 °C/min and finally held at 50 °C for 5 min.

Water holding capacity

Water holding capacity (WHC) was determined using the method of Chun-He, Ten, Xian-Sheng and Xiao-Quan (2006) with slight modification described in the work of Kain, Chen, Sonda and Abu-Kpawoh (2009). The results are presented as g of water per g of product.

Statistical analysis

Significant difference test were used to analyze variations of the results. Differences between the means with probability $P < 0.05$ were accepted as statistically significant. The level of confidence was set at 95% (STATISTICA (Data Analysis Software System), 13.0, TIBCO Software, USA Stat-Soft, Inc, USA, www. statsoft.com)**.**

RESULTS AND DISCUSSION

Three carp feed formulations with same starch content (approximately 230 g/kg) and particle size as shown in Table 1 were evaluated for their rheological properties using a Mixolab device as well as their water absorption properties (Fig. 1).

Results of rheological assessment were shown in Table 2. In Figure 2 Mixolab curves are presented for the investigated diets. During the initial stage of mixing at 30 °C, hydration of the mix compounds occurs, accompanied by the stretching and alignment of proteins, resulting in the formation of a viscoelastic dough structure (Rosell, Collar & Haros, 2007). Water absorption was set at the constant level of 100% and increase in torque (C1) was observed until it reached the maximum value of 0.22 Nm, 0.85 Nm and 0.39 Nm for control, formulation 1 and formulation 2, respectively.

During the second stage, torque decreased to a minimum due to the weakening of the protein network under mechanical shear stress and heat. As the temperature further rises, starch gelatinization becomes the primary factor affecting torque. Starch granules absorb water, swell, and release amylose into the surrounding medium, increasing viscosity and torque. This process continues until shear stress and heat induce granule breakdown leading to a reduction in viscosity (Rosell et al., 2007). A decrease in temperature led to an increase in torque, known as setback, which corresponds to the cooling and gelation process. The final stage involves retrogradation (Tran et al., 2020). The main protein source (fishmeal) was completely replaced in F1 with novel plant protein source, duckweed powder, with the inclusion level of 200 g/kg and this mix showed significantly higher torque value (C1) compared to the control, exhibiting greater resistance to deformation. Moreover, F2 did not exhibit significant differences in the C1 parameter compared to control, despite the complete substitution of soybean meal with duckweed. These results were in accordance with the results of WHC (Fig.1), which indicates much greater capacity in water binding of F1 compared to control and diet 2 (Fig.1).

Table 2.
Rheological characteristics of carp feed formulations

Hydration % (14% base)			
Parameter	**Control**	**F1**	**F2**
Water absorption (%)	100	100	100
Moisture content (%)	6.25	6.50	6.90
C1 (Nm)	0.22^{a}	0.85^{b}	0.39^{a}
Dough development time (min)	10.36^{a}	10.20^{a}	9.83^{a}
C2 (Nm)	0.14^{a}	0.56^{b}	0.26^{a}
TC2 (min)	21.58^{a}	20.91^{a}	27.19^{a}
C3 (Nm)	0.42^{a}	0.94^{b}	0.46^{c}
TC3 (min)	26.19^{a}	23.99^{b}	25.15^{c}
C4 (Nm)	0.40^{ab}	0.44^{b}	0.35^{a}
TC4 (min)	33.27^{a}	31.93^{a}	32.15^{a}
C5 (Nm)	0.52^{a}	0.65^{b}	0.53^{a}
TC5 (min)	45.02	45.02	45.02
C5-C4 (Nm)	0.12^{a}	0.21^{b}	0.18^{b}
C3-C4 (Nm)	0.02^{a}	0.45^{b}	0.11^{c}
C1-C2 (Nm)	0.08^{a}	0.29^{b}	0.13^{c}

Each value in the table was the mean of two replications. $^{a-c}$ Sample means with different superscript letters in the same row are significantly different at $p<0.05$.

C1 - Maximum torque during mixing (Nm), used constant hydration of 100%; C2 - Minimum consistency (Nm), the lowest torque value during the dough's exposure to mechanical and thermal constraints (protein weakening stage); C3 - Peak torque (Nm), the peak torque generated during the heating phase, indicating the rate of starch gelatinization; C4 - Minimum torque (Nm) during the heating phase, reflecting the stability of the hot gel formed; C5 - Final torque (Nm), the torque after cooling at 50 °C, representing starch retrogradation during the cooling stage; C5-C4 - Setback torque (Nm) - anti-staling effects (starch retrogradation at cooling stage), representing the shelf-life of the end products (Schmiele, Felisberto, Clerici & Chang., 2017); C3-C4 -Breakdown torque (Nm) - speed starch gelatinization; C1–C2 - Attenuation rate of protein in warming (Konvalina et al., 2017)

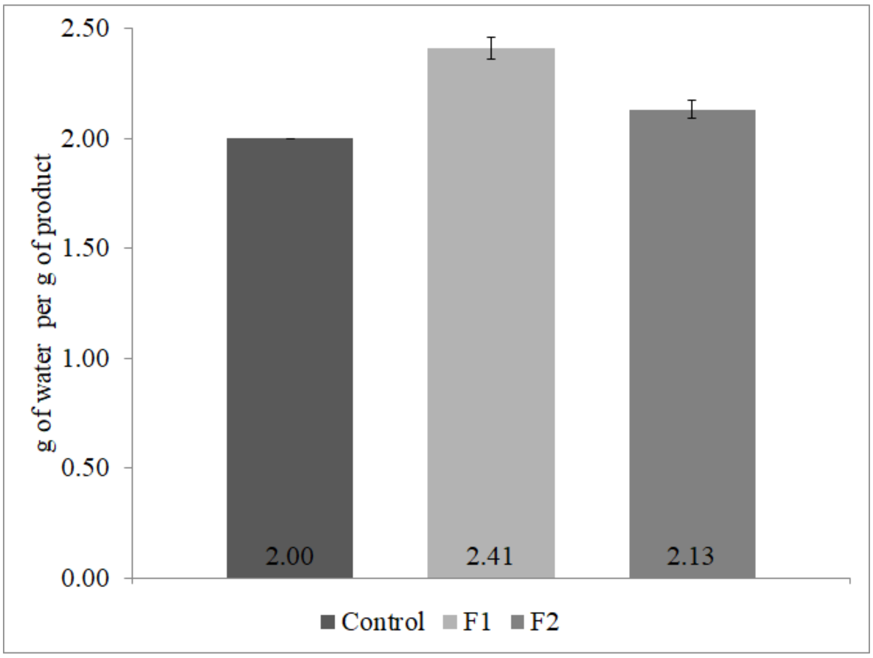

Figure 1. Water holding capacity of carp feed mixtures

Increased water absorption properties of the F1 as well as C1 value can be related to the lower lipid content (58.3%) (Table 2) in comparison to control (67.90%) and F2 formulation (73.70%). Lipids can act as lubricant and can prevent water absorption by other ingredients. Furthermore, the reason for increased WHC of the sample containing duckweed could be related to high content of hydrophilic compounds which can be found in duckweed, such as proteins, polysaccharides (like cellulose and hemicellulose), and fibers (Xu et al., 2023; Appenroth et al., 2017; Gwaze & Mwale, 2015). F1 also expressed significantly lower weakening of proteins due to mechanical work and temperature (parameter C2) compared to the control and F2 which can be attributed to the lower protein content. F1 exhibited significantly higher value of torque during the heating stage (parameter C3) compared to the control and F2, expressing the greater rate of starch gelatinization compared to other two mixture which can be ascribed to lower fat content. It was already proved that fat interacts with starch components by building amylose-lipid complexes which influence lower peak viscosities and delayed starch gelatinization (TC3 parameter) (Codină, Istrate, Gontariu & Mironeasa, 2019). Furthermore, this reduction in free amylose content limits intermolecular association during cooling resulting in delayed starch retrogradation (C5-C4 value). During the stage of physical breakdown of starch granules, which leads to reduction in viscosity, minimum torque values (C4) are observed. Control did not show any significant differences compared to other two mixtures, while between F1 and F2 it is observed that F1 has significantly greater hot gel stability. F1 had significantly higher starch retrogradation value (C5) during the cooling phase compared to control and F2. Control had the lowest final consistency and setback (C5-C4) value. Significant differences between F1 and F2 had not been observed. Breakdown torque (C3-C4) had the lowest value in control and highest in F1 mainly due to higher fat content of control and F2 formulations. As it was mentioned previously, higher fat content is responsible for surface coverage and amylose-lipid complexes which restrict swelling and starch gelatinization. Therefore, the peak viscosity was the highest for F1 formulation, characterized with the lowest fat content. Consequently, decrease in torque value C4 and also C3-C4 value upon further mixing was more pronounced for F1 formulation. Further mixing at maximum temperature did not have such a pronounced effect in other formulations due to the presence of fats which prevented the starch from gelatinizing as strongly as in the F1 sample. The practical significance of these results indicates the behavior of mixtures during steam conditioning process that normally precede to the extrusion processing in contemporary fish feed production. Also, the results can give insight in how the mixture would act during low-moisture extrusion process and how the properties of the different formulations (such as water absorption, fat content, and starch behavior) impact the quality of the resulting fish feed pellets. Extrusion cooking is a complex process that combines heat and mechanical forces. During the process, the mixture is exposed to moisture, high temperatures, shear forces, and pressure, which cause complex physical, chemical, and biological transformations of material, such as starch gelatinization, protein unfolding, denaturation, aggregation, and the formation of disulfide bonds. Such modifications occur at both the macroscopic level - affecting expansion, texture, and overall appearance - and the microscopic level, altering molecular structures and the formation of covalent and non-covalent bonds. The substitution of fishmeal with duckweed powder in formulation F1 resulted in higher WHC and greater resistance to deformation (C1), which suggested that this formulation may contribute to producing more stable and homogeneous fish pellets during the extrusion process. Additionally, the lower fat content of F1, which has a direct influence on the interaction between starch and water, resulted in higher starch gelatinization and gel stability. This characteristic is important for the extrusion process, as it ensures better pellet integrity, preventing the pellets from breaking down during storage or feeding. The comparison between F1 and F2 revealed that F1, with the lower fat content, demonstrated superior performance in terms of starch gelatinization and retrogradation (C3 and C5 parameters), which are critical for producing durable fish pellets. The increased gel stability and starch retrogradation observed in F1, compared to the control and F2, suggested that this formulation could lead to more robust pellets, minimizing the loss of solid material and ensuring higher durability during feeding (Liu, Frost, Welker & Barrows, 2021). Also, the obtained results mean that F1 mixture could be conditioned and processed via extrusion at lower temperatures than control mixture, at same moisture content. On the other hand, if steam conditioning of these mixtures prior the extrusion is done at the same condition temperatures, the higher level of moisture must be achieved when duckweed is present in mixture. The reason for this is the fact that the plant proteins absorb more quickly water compared to the starch rich grains, thus competition for moisture within different ingredients can occur and grains will not have adequate moisture level for achieving proper gelatinization of starch.

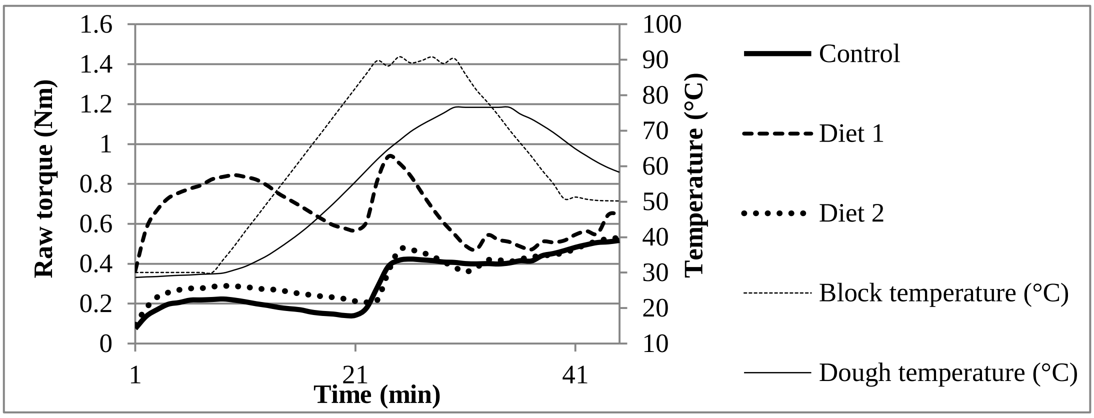

Figure 2. Mixolab profile

CONCLUSIONS

The obtained results highlighted the significant impact of ingredient composition on the functional properties of feed formulations during thermo-mechanical processing. Variations in protein and fat content influenced water absorption, starch gelatinization, and gel stability as determined by Mixolab, all of which are critical factors in determining the final product texture and durability during extrusion processing. Lower fat content was associated with increased water absorption and enhanced starch gelatinization, which could indicate improved fish pellet integrity. The substitution of fishmeal with duckweed powder significantly influenced the rheological behavior of the mixture during processing. Formulation F1 exhibited higher water absorption capacity, greater resistance to deformation superior starch gelatinization and retrogradation, which are the key factors for producing durable and structurally stable fish pellets. Based on the present results, it is demonstrated that Mixolab is valuable tool for evaluating the functionality of fish feed mixtures, particularly in terms of their behavior during critical processing stages like mixing, hydration, and heating. A comprehensive understanding of the properties and interactions of various feed ingredients in mixtures, along with their thermo-mechanical characteristics, can provide valuable insights into their behavior during different processing stages, such as conditioning and extrusion. This knowledge can, in turn, lead to more effective process management in fish feed production.

AUTHOR CONTRIBUTIONS

Conceptualization, V.B..; Methodology, P.I., V.B and M.H.; Investigation, formal analysis, validation, P.I., V.B., M.H., S.V and V.S.; Writing-original draft preparation, P.I.; Writing-review and editing, V.B., M.P. and M.H.; Supervision, M.P. and B.T.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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ODREĐIVANJE REOLOŠKIH SVOJSTAVA SMEŠA HRANE ZA ŠARANA SA DODATKOM RAZLIČITIH PROTEINSKIH SIROVINA KORIŠĆENJEM MIKSOLABA

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Sažetak: Za potrebe ovog istraživanja formulisane su tri smeše za ishranu šarana sa istim sadržajem skroba (oko 230 g/kg) i sa dodatkom proteinskih sirovina različitog porekla. Kontrolna smeša sadržala je riblje brašno i sojinu sačmu kao glavne izvore proteina, koji su potom zamenjeni sušenom sočivicom. Svi sastojci smeše su fino samleveni mlinom čekićarem, a potom su umešani. Cilj ovog istraživanja bio je određivanje reoloških i termo-mehaničkih osobina smeša kako bi se predvidelo njihovo ponašanje tokom dalje obrade. Određivanje reoloških osobina postignuto je pomoću miksolaba gde je kapacitet apsorpcije vode bio podešen na konstantnu vrednost od 100%. Očitani parametri su se razlikovali, gde je smeša u kojoj je riblje brašno u potpunosti zamenjeno novom proteinskom sirovinom biljnog porekla, brašnom od sočivice, pokazala najveći otpor ka deformaciji, prema tome i najveću sposobnost upijanja vode, što je u saglasnosti sa rezultatima dobijenim primenom metode za određivanje moći upijanja vode. Dobijeni rezultati su pokazali da miksolab poseduje dobar potencijal kao alat za korišćenje u svrhu određivanja neophodne količine vode i pare tokom vođenja procesa kondicioniranja. Dalja istraživanja su potrebna kako bi se povećala primenljivost ovih metoda.

Ključne reči: *hrana za ribe, miksolab, reologija skroba*

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