

A Case Study on Maximizing Aqua Feed Pellet Properties Using Response Surface Methodology and Genetic Algorithm

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ABSTRACT

Aims: The present case study is on maximizing the aqua feed properties using response surface methodology and genetic algorithm.

Study design: Effect of extrusion process variables like screw speed, L/D ratio, barrel temperature, and feed moisture content were analyzed to maximize the aqua feed properties like water stability, true density, and expansion ratio.

Place and Duration of Study: This study was carried out in the Department of Agricultural and Food Engineering, Indian Institute of Technology, Kharagpur, India.

Methodology: A variable length single screw extruder was used in the study. The process variables selected were screw speed (rpm), length-to-diameter (L/D) ratio, barrel temperature (°C), and feed moisture content (%). The pelletized aqua feed was analyzed for physical properties like water stability (WS), true density (TD), and expansion ratio (ER). Extrusion experimental data was collected by based on central composite design. The experimental data was further analyzed using response surface methodology (RSM) and genetic algorithm (GA) for maximizing feed properties.

Results: Regression equations developed for the experimental data has adequately described the effect of process variables on the physical properties with coefficient of determination values (R^2) of > 0.95 . RSM analysis indicated WS, ER, and TD were maximized at L/D ratio of 12-13, screw speed of 60-80 rpm, feed moisture content of 30-40%, and barrel temperature of $\leq 80^\circ\text{C}$ for ER and TD and $> 90^\circ\text{C}$ for WS. Based on GA analysis, a maximum WS of 98.10% was predicted at a screw speed of 96.71 rpm, L/D ratio of 13.67, barrel temperature of 96.26°C , and feed moisture content of 33.55%. Maximum ER and TD of 0.99 and 1346.9 kg/m^3 was also predicted at screw speed of 60.37 and 90.24 rpm, L/D ratio of 12.18 and 13.52, barrel temperature of 68.50 and 64.88°C , and medium feed moisture content of 33.61 and 38.36%.

Conclusion: The present data analysis indicated that WS is mainly governed by barrel temperature and feed moisture content, which might have resulted in formation of starch-protein complexes due to denaturation of protein and gelatinization of starch. Screw speed coupled with temperature and feed moisture content controlled the ER and TD values. Higher screw speeds might have reduced the viscosity of the feed dough resulting in higher TD and lower ER values. Based on RSM and GA analysis screw speed, barrel temperature and feed moisture content were the interacting process variables influencing maximum WS followed by ER and TD.

Keywords: Extrusion cooking, aqua feed properties, response surface methodology, genetic algorithm

1. INTRODUCTION

Extrusion is a very popular and important processing operation in the food industry to produce novel products (Riaz, 2012; Paton and Spratt, 1984). Extrusion cooking is a high temperature and short-time process used for production products like snack foods, ready-eat-cereals, animal feeds, and pet foods (Chinnaswamy and Hanna, 1988). During extrusion, raw material is cooked and plastized in the presence of moisture, temperature, and mechanical shear resulting in texturized novel products. During World War II, corn snacks were commercially produced using high-shear extruders. In the last five decades, food extrusion has developed from a simple pressing and forming technology into a sophisticated cooking process, and has replaced many conventional food processing technologies (Ilo et al., 2000). The major advantages of pelletization of foods and feeds using extrusion are a) improved digestibility due to gelatinization of starch; b) ingredient separation prevention during handling and transit; and c) the physical integrity and chemical composition of the pellets are maintained for extended periods during storage, handling, and transportation (Harper 1981; Paton and Spratt 1984; Chinnaswamy and Hanna, 1988). According to Singh et al. (2007), the beneficial effects of extrusion cooking include the reduction of anti-nutritional factors, the gelatinization of starch, an increase in soluble dietary fiber, and a reduction of lipid oxidation, whereas the detrimental effects include changes in amino acid profile, carbohydrates, dietary fiber, vitamins, and mineral content. Extrusion process variables like moisture, pressure, and mechanical shear partially denature the protein and gelatinize the starch components of the feed. These chemical modifications alter the texture resulting in physiochemical changes in the extruded products (Friesen et al., 1992; Bhattacharya and Prakash, 1992; Camire et al., 1990; Athar et al., 2006; Dar et al., 2012). Reactions like gelatinization of starch and denaturing of protein of the multi-component feed mixes result in producing feed pellets with higher water stability, expansion ratio, bulk density, and durability (Harper, 1986; Lim and Cuzon, 1994).

1.1 Aqua Feed Physical Properties

Knowledge of the physical properties and process variables influencing them are essential for producing high quality aqua feed pellets, designing equipment for processing facilities, and optimizing unit operations (Rosentrater, 2006). Some key quality attributes of aqua feed pellets are water stability; bulk density; moisture content; water activity; yield stress; apparent viscosity; thermal conductivity; thermal diffusivity; heat capacity; drying behavior; and mass, bulk, and unit densities (Rosentrater and Muthukumarappan, 2006). Extrusion helps to manufacture pellets from multi-component aqua feed mixes with both animal (e.g., fish meal, squid meal, shrimp head meal, and low-cost fishes) and plant sources (e.g., de-oiled soy meal, de-oiled rice bran, and wheat flour) with desirable physical properties like high water stability (WS), expansion ratio (ER), and true density (TD). According to Wood (1995), selecting the right process conditions and inclusion of ingredients play a major role on the quality attributes of the aqua feed pellets. WS is influenced by the binding ability of the ingredients, the size of those ingredients, and extrusion process conditions. Effiong et al. (2009) indicated that feed ingredients have a great impact on the water stability of the pellets. In their studies on extrusion processing of aqua feed mix to produce pellets with high WS values, both Rout and Bandyopadhyay (1999) and Bandyopadhyay and Rout (2001) indicated that reducing the size of the ingredients during extrusion can also result in high WS as the air space between the particles is reduced and prevent water or moisture to seep through the fine cracks of the pellets. In general, lower WS values results in the disintegration of pellets quickly and facilitates faster leaching of the nutrients and micronutrients, which undergo an oxidation-reduction process causing water pollution and deposition of peat and muck in the fish pond bottom (Lim and Cuzon, 1994; Ali, 1988). According to Ali et al. (2005), aqua feed water stability is achieved mainly through the use of appropriate binding material coupled with a suitable processing technology.

Two additional quality attributes—expansion ratio (ER) and true density (TD)—are equally important as they increase digestibility and prevent drifting of aqua feed pellets by air or waves during feeding (Rout, 1997). Chevanan et al. (2009) has used novel ingredients like distilled dried grains with solubles and whey to improve extrudate properties like durability and density. They have also found that feed moisture content and extruder screw speed significantly impact the extrudate properties. In their studies on the physical behavior of feed pellets in Mediterranean waters, Vassallo et al. (2006) indicated that the length of the pellets extruded affects the settling velocity. Kannadhasan et al. (2011) studied the effect of starch sources and protein on the physical properties of aquaculture feed containing distilled dried grains with soluble. These researchers concluded that extrudate properties such as expansion ratio, unit density, sinking velocity, color, water absorption, solubility indices, and pellet durability index are an important factor in checking the suitability of feed for various fish species. These properties are dependent on the ingredients used in the feed preparation. Rolfe et al. (2001) concluded that moisture content of the feed, particle size, and screw speed effect properties like durability and water stability. Kraugerud et al. (2011) concluded that physical properties of extruded fish feed are strongly related to the starch content, especially the expansion ratio. They further point out that adding cellulose to feed diets resulted in negative correlation with ER values. Meanwhile, Garg and Singh (2010) concluded that expansion ratio and the bulk density of extruded soy-rice blend extrudates are affected by barrel temperature, feed moisture content, and soy flour-rice blend ratio.

1.3 Response Surface Methodology and Genetic Algorithm

Response surface methodology (RSM) is a combination of mathematical and statistical techniques widely used in product development (Khuri and Cornell, 1987). Many researchers (Frazier et al., 1983; Bhattacharya and Prakash, 1994; Rout and Bandyopadhyay, 1999; Sacchetti et al., 2004; Shankar and Bandyopadhyay, 2004 and 2005; Zhang and Gao, 2007; Wang et al., 2009; Shankar et al., 2010a; Bhattacharya et al., 2011; Tumuluru et al., 2013; Lopes et al., 2012; Sobukola et al., 2012; Minf et al., 2012) have used RSM to understand the effect of process variables on product characteristics. In particular, Shankar et al. (2010a) state that RSM is a good approach to summarize the trends of process variables for either maximization or minimization of the product quality. These same authors also indicate that the interpretation of RSM results is very complex, especially when optimizing a function with more than three independent variables. In general, more than three variables results in optimum values tuning out to be saddle points.

To understand real time and complex processes like extrusion and back propagation algorithms for prediction and genetic algorithms (GAs) for optimization have gained importance (Shankar and Bandyopadhyay, 2004 and 2007; Chen and Ramaswamy, 2002; Koc et al., 2007; Shankar et al., 2010a, Mokeddem and Khellaf, 2009; Enitan and Adeyemo, 2011; Babakar and Akandi, 2012). GAs finds extensive application where the process systems are highly complex and nonlinear (Deb, 2000; Holland, 1992). Chun et al. (1998) discussed the usefulness of heuristic algorithms as the search method for diverse optimization problems. Their studies include comparison of immune algorithms, genetic algorithms, and evolutionary algorithms on diverse optimization problems and indicated that results of genetic algorithm are superior to others. Using RSM and GA collectively for chemical process data analysis can help to overcome the limitation of RSM and reach the global optimize process conditions for the desired product properties. In fact, Shankar et al. (2010a) have successfully used RSM and GA in combination for optimizing the biomass flow in a single screw extruder.

1.4 Objective

Aqua feed formulation mix is extremely complex as a variety of raw materials from both animal and plant sources are used. Effect of extrusion process variables like barrel

temperature, L/D ratio, feed moisture content, and screw speed on multi-component high protein, fat, and fiber-rich feed formulation is very complex as well. Published literature on using response surface methodology and genetic algorithm on the analysis of aqua feed physical properties data is not available. The specific objectives of the present research are a) to understand the effect of extrusion process variables like screw speed (rpm), L/D ratio, barrel temperature (°C) and feed moisture content (%) on aqua feed pellet properties such as water stability (WS), expansion ratio (ER), and true density (TD) using response surface plots; and b) to optimize the process conditions using GA for the maximum limits of WS, ER, and TD, respectively.

2. MATERIAL AND METHODS

2.1 Experimental Data

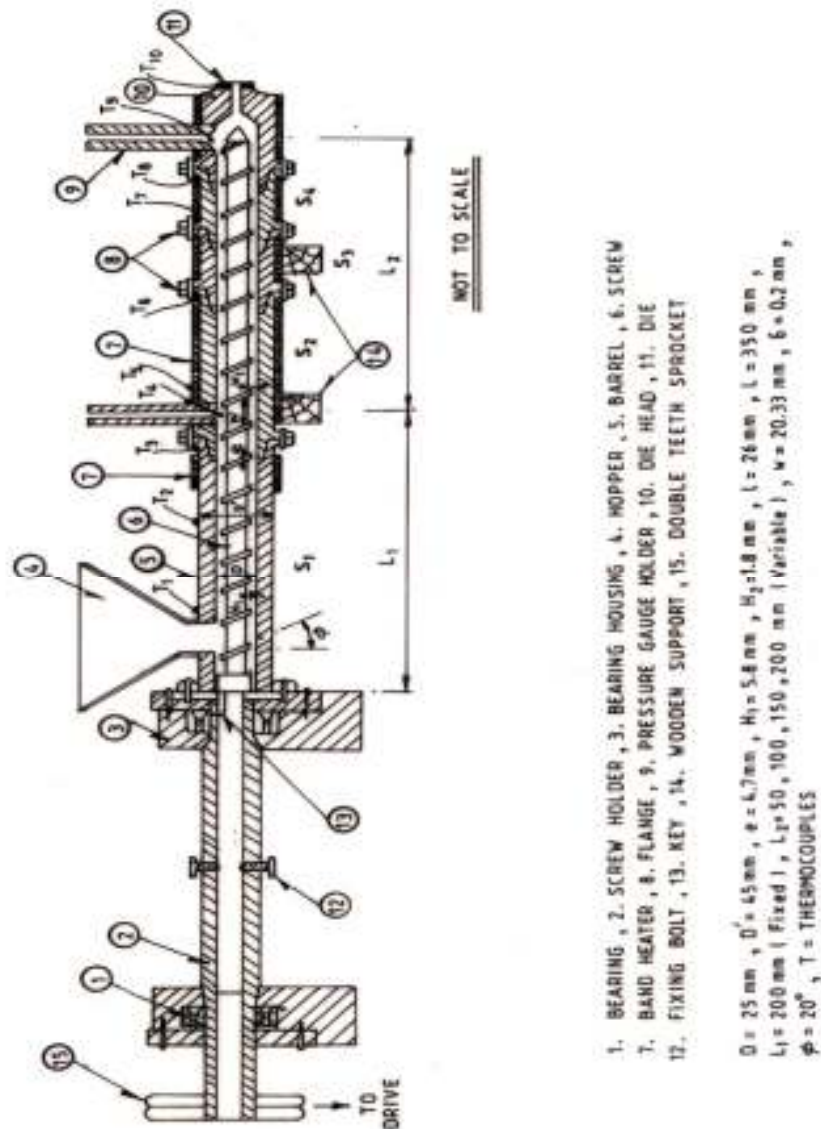
Experimental data on aqua feed pellet properties with respect to process variables like screw speed, L/D ratio, barrel temperature, and feed moisture content was adapted from the studies conducted by Rout (1997), Rout and Bandyopadhyay (1999), and Tumuluru et al. (2013). Figure 1 indicates the design features of the variable length single screw extruder used (Rout, 1997; Rout and Bandyopadhyay, 1999; and Tumuluru et al., 2013). Rout and Bandyopadhyay (1999) and Bandyopadhyay and Rout (2001) conducted experiments at five levels, as shown in Table 1. A least-cost formula based on the simplex method was used to design the multi-component feed extrudate for *Penaeus monodon*. Table 2 indicates the list of ingredients used in the aqua feed mix. The raw material is initially ground to 0.3 mm particle size, and the dough is prepared by adding the required quantity of water to the feed mix based on the experimental design. The mixed feed dough is kept in a polythene bag for 10 minutes to attain moisture distribution. Further, the feed mix dough is extruded at different levels of extrusion process variables based on the experimental design. During extrusion, only the steady state output is used for the physical properties measurement conducted by Rout (1997) and Shankar et al. (2010a).

Table 1. Five level central composite design (Rout, 1997).

Independent variables	Code	Coded levels				
		-2	-1	0	1	2
Screw speed (rev min ⁻¹)	x ₁	20	50	80	110	140
L/D ratio	x ₂	8	10	12	14	16
Barrel temperature (°C)	x ₃	60	70	80	90	100
Feed moisture content (%)	x ₄	20	30	40	50	60

Table 2. Composition of multi-component aqua feed mix (Rout, 1997).

Ingredients	% Inclusion
Fish meal	29.37
Shrimp head meal	10.00
Squid meal	5.00
Wheat flour	28.25
Deoiled rice bran	4.01
Deoiled soybean meal	15.00
Fish oil	2.27
Lecithin	1.00
Micro-ingredients	5.10



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158 Fig. 1. Schematic of the extruder used in the present study (Tumuluru et al., 2013).

159 2.2 Measurement of Aqua Feed Properties

160 Rout (1997) and Rout and Bandyopadhyay (1999) have discussed the experimental
 161 methods followed for the measurement of aqua feed properties like water stability (WS),
 162 expansion ratio (ER), and true density (TD) in detail. Expansion ratio is defined as the ratio
 163 of diameter of the dried pellets to that of the die diameter of the extruder. WS was measured
 164 using wire mesh baskets with a 0.5 mm opening. A batch of 20 g pellets were placed in
 165 these baskets and immersed in saline water. The top of the baskets was kept out of water
 166 surface whereas the feed was allowed to stay in water column for a period of 120 min. After
 167 the test period the samples were taken out of the baskets and dried in an oven at 100°C for

3 hours. WS is calculated by measuring the weight of dried sample left in the basket to the original weight. True density was measured using pycnometer or specific gravity bottle using toluene (Punima, 1985). The procedure includes measuring the mass of a) an empty specific bottle; b) a specific gravity bottle + feed pellets; c) a specific gravity bottle + feed pellets +toluene; d) a specific gravity bottle+ toluene; and e) a specific gravity bottle + distilled water. True density is calculated using Equation (1).

$$\rho_f \frac{\text{Dry mass of feed particles}}{\text{Mass of toluene of equal volume}} \times \rho_t \quad (1)$$

where ρ_f is the true density and ρ_t is the density of toluene.

2.3 Data Analysis

The experimental data generated by Rout (1997) and Rout and Bandyopadhyay (1999) was further analyzed using response surface methodology (RSM) and genetic algorithm (GA). These two powerful data analysis techniques are widely used for process modeling and optimization. RSM is a combination of statistical methods, whereas GA is an evolutionary algorithm, which optimizes function based on natural selection.

2.4 Response Surface Methodology

A second-order response model (see Equation 2) was chosen to develop the regression equations in original terms for WS, ER and TD.

$$y = b_0 + \sum_{i=1}^n b_i x_i + \sum_{i=1}^n b_{ii} x_i^2 + \sum_{i=1}^n \sum_{j=1}^n b_{ij} x_i x_j + \varepsilon \quad (2)$$

where y is the dependent variable, x_i and x_j are the coded independent variables, b_0 , b_i , and b_j are coefficients, n is the number of independent variables, and ε is an unobservable error. The significance of the linear, quadratic and interactive terms was evaluated based on the p-value. Regression equations (see Table 3) are further used for drawing the response surface plots and also for optimization using genetic algorithm. Statistica software Version 9.0 (Statsoft Inc., Tulsa, OK) was used for the statistical analysis and for drawing the response surface plots.

2.5 Genetic Algorithm

The working principle of genetic algorithms (GAs) is based on Darwinian's theory of survival of the fittest. In GA analysis, a random population is generated initially, which are normally the possible solutions of the objective function. The individuals in the population are represented by a string of symbols called chromosomes. Binary bit strings are used to represent the chromosomes. The length of the chromosome is selected based the precision desired. The chromosomes selection is based on the goodness of a chromosome in the population and is evaluated over a fitness function. A linear rank fitness selection method (see Equation 3) is widely used as it behaves in a robust way and helps to prevent the stocking of the population (Shankar et al., 2010a and b; Sivaraj, 2011).

$$f_i = 2 - SP + 2(SP - 1) \times \frac{(Pos - 1)}{(n - 1)} \quad (3)$$

where n = the number of individuals in the population, Pos is position of an individual in this population, and SP = selective pressure, which indicates the probability of selecting the best individual in the population.

Parent population of the fitted population is selected based on the Roulette Wheel selection technique (see Equation 4). In this process, chromosomes with the highest fitness values are selected, whereas the lower ones are eliminated. If the fitness of ' n ' individuals in a population is $f_1, f_2, f_3, \dots, f_n$, the selection probability (P_i) of an individual is calculated using this equation (Deb, 2000).

$$P_i = \frac{f_i}{(f_1 + f_2 + f_3 + \dots + f_n)} \quad (4)$$

The other two operations used in genetic algorithm are crossover and mutation. The crossover operation was carried out on the selected population based on the Roulette wheel selection. In crossover operation, bit strings of the selected parent chromosomes are exchanged to generate new populations called offspring. A single point crossover method was used in the present study. Mutation operation, which is a bit of inversion process, helps to a) maintain the diversity within the population; b) inhibit the premature convergence of the population; and c) prevent the population from getting stuck at local points. The steps used in genetic algorithm are given below (Shankar et al., 2010a).

Step 1: Random populations of chromosomes, which are suitable solutions for the problem.

Step 2: Fitness values are evaluated for the population generated.

Step 3: New population is generated using the step 2 till the population is complete.

Step 4: Two parent chromosomes are selected based on the fitness values. Higher the fitness values better the chance for the population to get selected as a parent.

Step 5: New parents are selected with certain amount of crossover probability where new offspring's are generated. In general, crossover probabilities of 85-95 % are recommended to prevent premature convergence. No crossover will generate offspring's, which are exact copy of the parents.

Step 6: The new offspring's generated are mutated (bit inversion) with certain amount of probability. Low mutation rate of about 0.5 percent to 1 percent is recommended to obtain optimized results from genetic algorithm. Small mutation rates prevent genetic algorithms from falling into local maxima or minima.

Step 7: New population generated is placed in the current population and used in the algorithm.

Step 9: If the end condition is satisfied (where fitness does not change after certain number of iterations), the algorithm is stopped and the best solution is returned to the population.

Step 10: Go to step 2.

The GA program developed by Shankar and Bandyopadhyay (2004) and Shankar et al. (2010a and b) was used in the present study. Regression equations developed for WS, ER, and TD were used as the objective functions. A random population of 100 chromosomes, crossover and fitness probabilities of 0.80 and 0.98, respectively, and 100 iterations were used. Figure 2 indicates the combination of RSM and GA used in the present study to understand the effect of extrusion process variables on aqua feed physical properties.

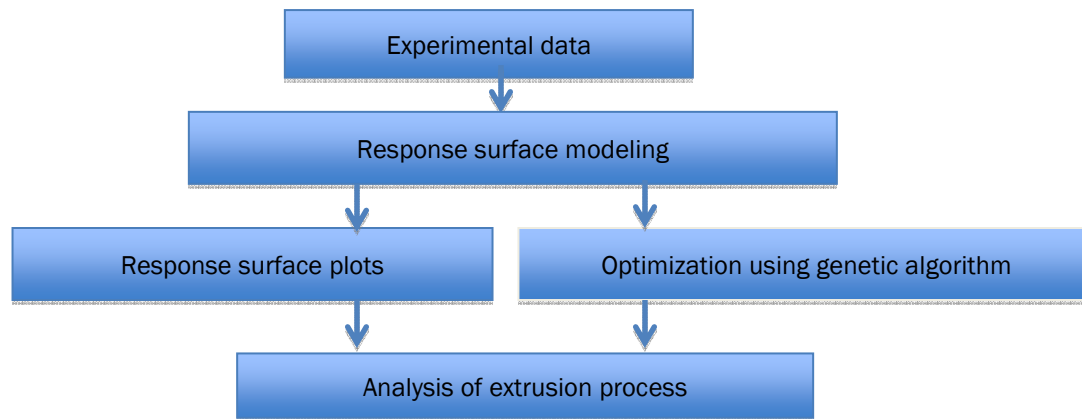


Fig. 2. Flow diagram for extrusion process data analysis using RSM and GA.

3. RESULTS

3.1 Response Surface Analysis

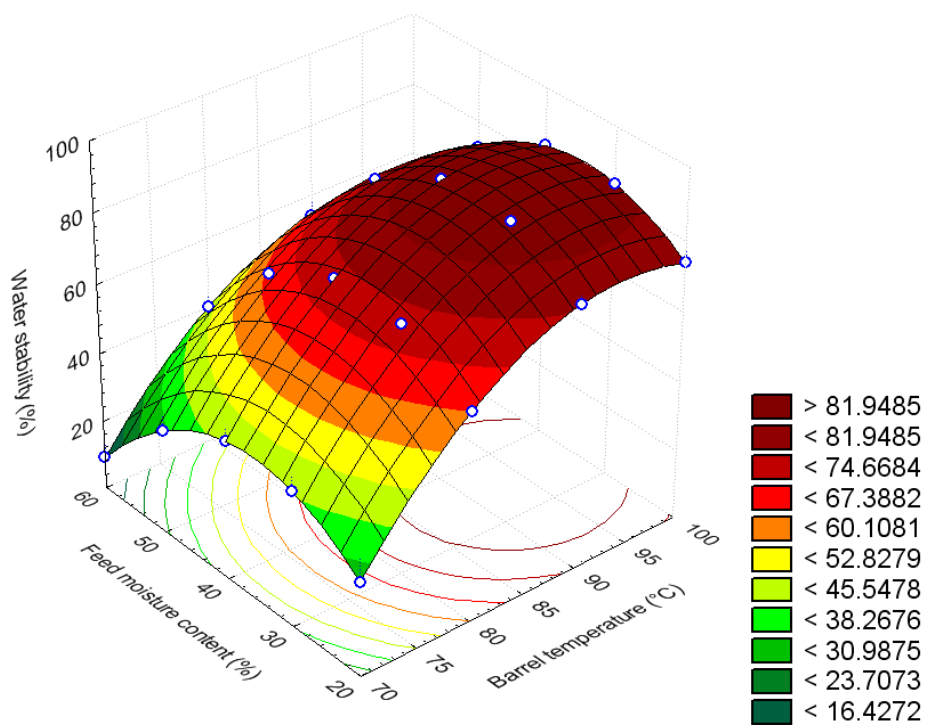
The coefficient of determination (R^2) values of the regression equations for WS, ER, and TD were 0.97, 0.95, and 0.95, respectively, indicating that they have adequately fitted. The predicted and observed plots drawn for WS, ER and TD (not shown) have corroborated with above observation.

Response surface plots were developed based on the regression equations (see Table 3) to understand the effect of extrusion process variables on WS, ER, and TD. Surface plots were drawn for each of the two variables, where the other two variables were kept at the center point of the experimental design. Some of the important surface plots are given in Figures 3–8. Surface plots (see Figures 3 and 4) indicated that barrel temperature $> 90^\circ\text{C}$, feed moisture content 30-40 %, L/D ratio of > 12 , and screw speed of 80 rpm maximized the WS values. Based on surface plots 5 and 6, the ER values are maximized at barrel temperature of $70\text{--}80^\circ\text{C}$, feed moisture content of 30-40%, screw speed of 60-70 rpm, and L/D ratio of 12. Surface plots of TD (see Figures 7 and 8) specify that a feed moisture content $< 40\%$, a barrel temperature $< 70^\circ\text{C}$, an L/D ratio of 12-14, and a screw speed of 80 rpm maximized the TD values. Table 4 indicates the summary of the trends of the extrusion process variables based on response surface plots for maximization of WS, ER, and TD values.

Table 3. Regression equations developed for WS, ER, and TD.

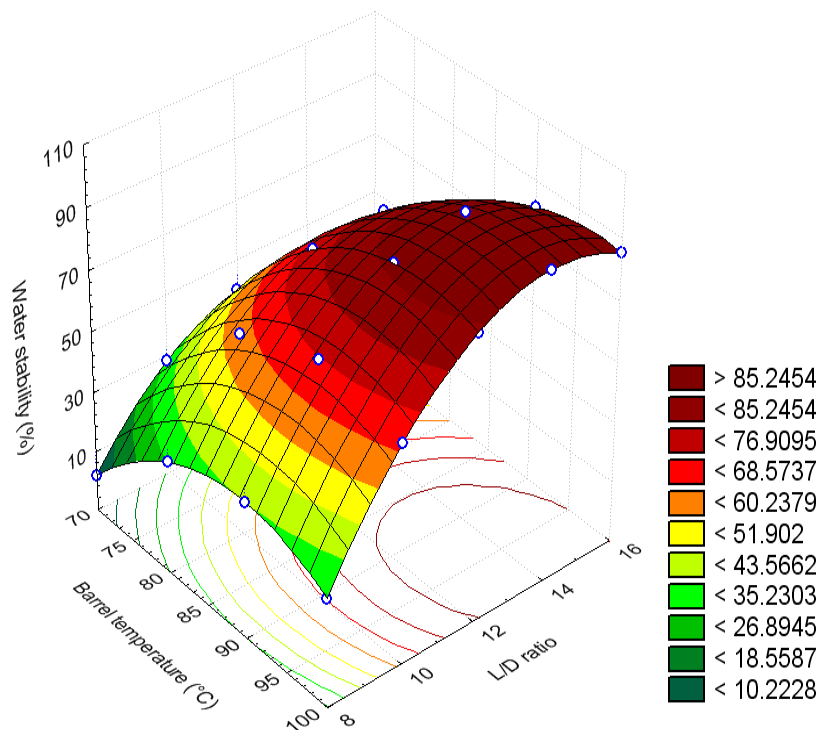
Aqua feed pellet property	Regression equations	(R^2)
WS (%)	$-914.521 + 0.566x_1 + 32.368x_2 + 14.57x_3 + 3.78x_4 - 0.0025x_1^2 - 1.78x_2^2 - 0.087x_3^2 - 0.055x_4^2 + 0.165x_2x_3$	0.97
Expansion ratio	$-0.345 + 0.0016x_1 + 0.0098x_3 + 0.015x_4 - 0.000018x_1^2 - 0.0042x_2^2 - 0.000118x_3^2 - 0.000192x_4^2 - 0.000155x_1x_2 + 0.000044x_1x_3 + 0.000253x_2x_3 - 0.000018x_1x_4 - 0.000384x_2x_4 + 0.000049x_3x_4$	0.95
True density (kg/m^3)	$560.4987 + 0.454x_1 + 57.89x_2 + 9.10x_3 + 3.62x_4 - 0.01x_1^2 - 2.83x_2^2 - 0.051x_3^2 - 0.045x_4^2 + 0.0912x_1x_2 + 0.300x_2x_4 - 0.067x_3x_4$	0.95

x_1 = screw speed (rpm), x_2 = L/D ratio, x_3 = barrel temperature ($^\circ\text{C}$) and x_4 = feed moisture content (%).



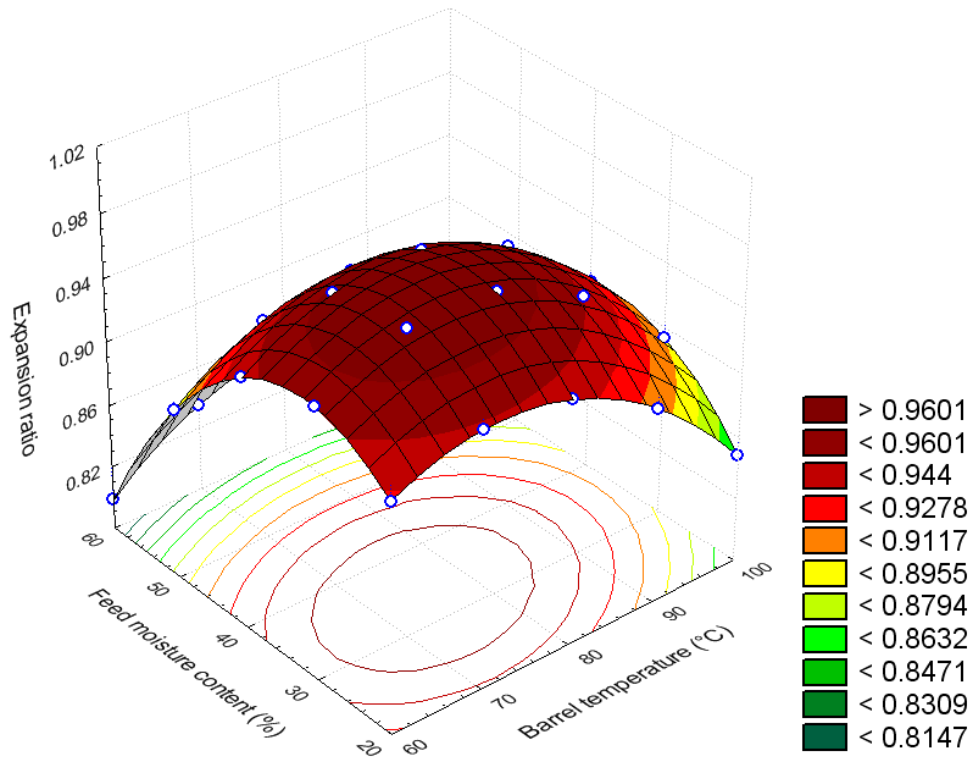
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Fig. 3. Surface plot of WS at screw speed of 80 rpm and L/D ratio of 12.



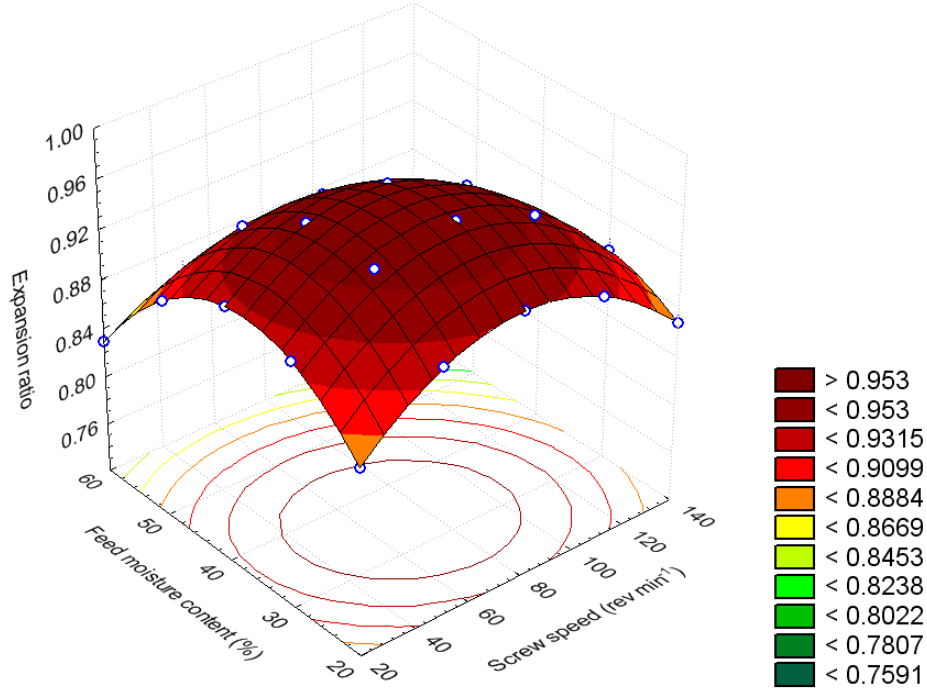
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Fig. 4. Surface plot of WS at screw speed of 80 rpm and feed moisture content of 40%.



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Fig. 5. Surface plot of ER at L/D ratio of 12 and screw speed of 80 rpm.



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Fig. 6. Surface plot of ER at L/D ratio of 12 and barrel temperature of 80°C.

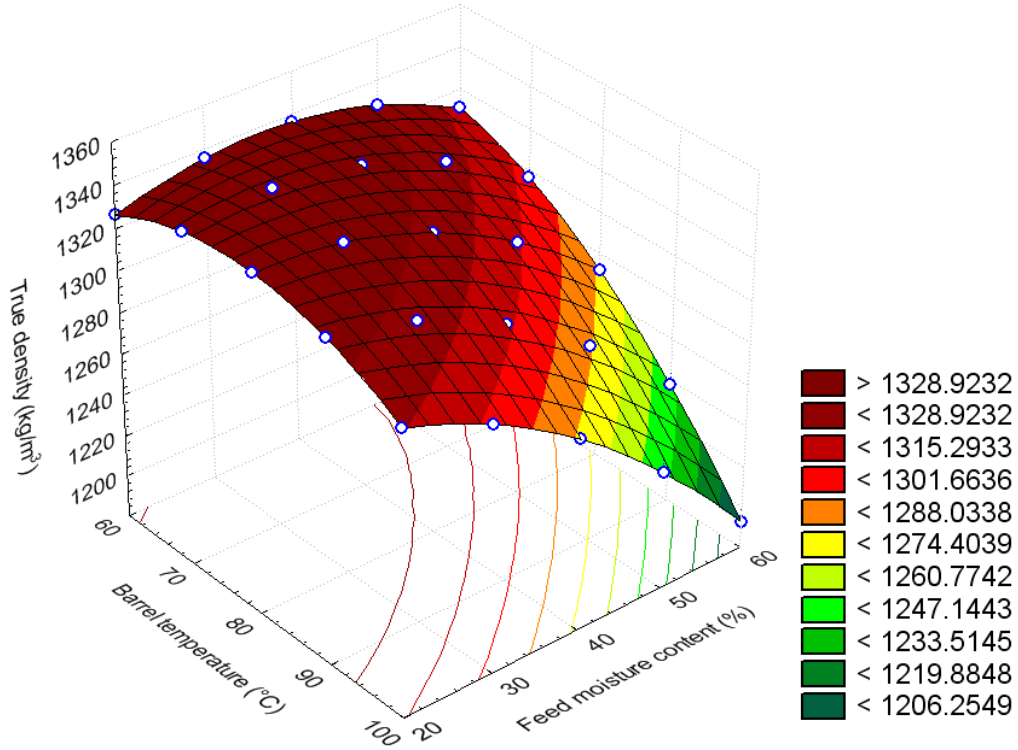


Fig. 7. Surface plot of TD at screw speed of 80 rpm and L/D ratio of 12.

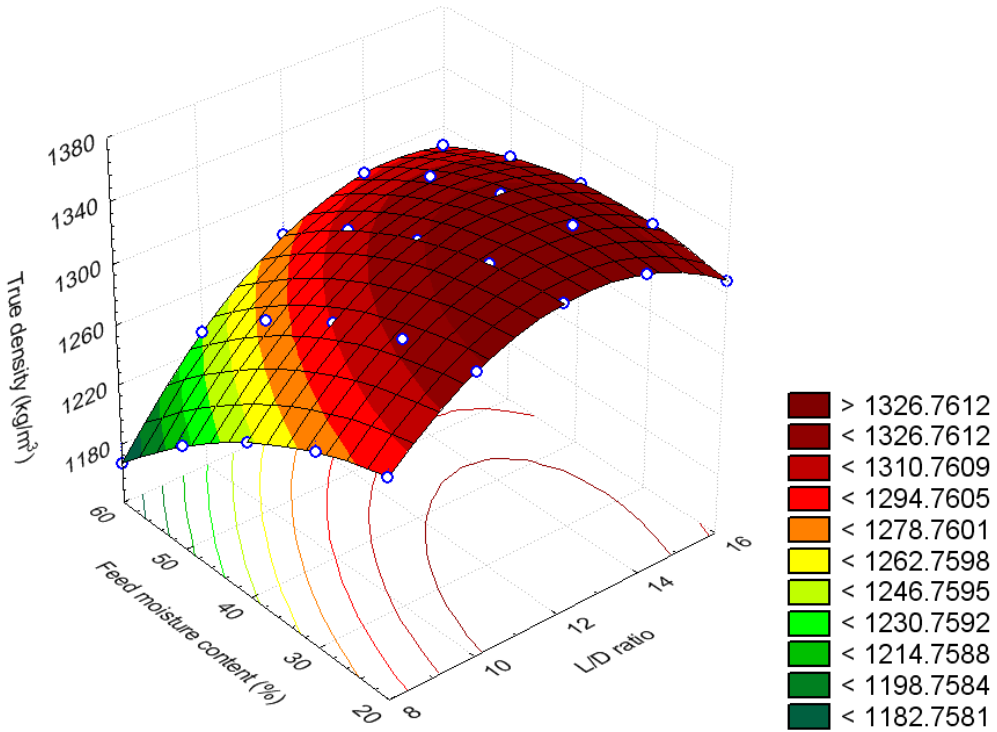


Fig. 8. Surface plot of TD at screw speed of 80 rpm and barrel temperature of 80°C.

Table 4. Summary of the extrusion process variables based on RSM.

Aqua feed pellet property	Extrusion process variables				Objective
	Screw speed, rev min ⁻¹ (x ₁)	L/D ratio (x ₂)	Barrel temperature, °C (x ₃)	Feed moisture content, % (x ₄)	
WS (%)	80	>12	> 90	30-40	Maximize
ER	60-70	12	70-80	30-40	Maximize
TD (kg/m ³)	80	>12	< 70	< 40	Maximize

3.2 Genetic Algorithm Analysis

Table 5 shows the optimum process conditions obtained using GA for maximization of WS, ER and TD using the response surface models. Maximum WS of 98.12 % was predicted at a screw speed of 89.90 rpm, an L/D ratio of 13.89, a barrel temperature of 94.34°C, and a feed moisture content of 36.01%. A screw speed of 60.37 rpm, an L/D ratio of 12.18, a low barrel temperature of 68.50, and a medium feed moisture content of 33.61% predicted a maximum ER value of 0.99, while a maximum true density of 1346.9 kg/m³ was predicted at a screw speed of 90.24 rpm, an L/D ratio of 13.52, a low barrel temperature of 64.88°C, and a medium moisture content of 38.36%.

Table 5. Optimum extrusion process variables based on genetic algorithm

Aqua feed pellet property	Optimum extrusion process variables				Maximum predicted pellet property
	Screw speed, rpm (x ₁)	L/D ratio (x ₂)	Barrel temperature, °C (x ₃)	Feed moisture content, % (x ₄)	
WS (%)	96.71	13.67	96.26	33.55	98.12
ER	60.37	12.18	68.50	33.61	0.99
TD (kg/m ³)	90.24	13.52	64.88	38.36	1346.9

4. DISCUSSION ON EXTRUSION PROCESS BASED ON RSM AND GA ANALYSIS

4.1 Water Stability (WS)

RSM and GA-based analysis of extrusion data indicated that L/D ratio, barrel temperature, and moisture content have a strong influence on WS aqua feed extrudate values. Mainly the gelatinization of starch and denaturation of protein present in the multi-component aqua feed mix and formation of starch-protein matrix govern WS of aqua feed pellets. Transformation of water insoluble starches into viscous gels due to gelatinization and the simultaneous conversion of soluble proteins into an insoluble fibrous network influence the WS values. Gelatinization primarily depends on the time-temperature relationship of the cooking process (Abdelrahman and Hosene, 1984; Venugopal and Keshavanath, 1984; Tacon, 1990). Many researchers have demonstrated that the extent of starch gelatinization is a strong function of barrel temperature and feed moisture content during extrusion of high starch food/feed materials (Bhattacharya and Hanna, 1987; Cai and Diosady, 1993; Chiang and Johnson, 1977; Colonna et al., 1984; Diosady et al., 1985; Guy and Horne, 1988; Lawton et al., 1972; Shankar and Bandyopadhyay, 2004). In the present study, the aqua

feed mix might have undergone the gelatinization of starch and resulted in the formation of a starch-protein matrix at high temperatures of >90°C. An L/D ratio coupled with screw speed and feed moisture content also had a significant effect on WS. These process variables provided the necessary heating surface and residence time for cooking, and might have resulted in formation of an insoluble fibrous network. Rockey and Plattner (2003) indicated that starch gelatinization at high temperatures and moistures during extrusion are crucial because it affects feed digestibility, expansion and contributes to water stability. They also indicated that the amount of starch gelatinized during processing depends on the starch type, particle size and processing conditions. In their studies on the pelleting of **distilled dried grains with solubles (DDGS)**, Tumuluru et al. (2010) observed that durability increased correspondingly with an increase in temperature and steam addition due to gelatinization of starch. Misra et al. (2002) in their studies on physiochemical parameter of aqua feeds like water stability are higher for extruded aqua feed pellets.

4.2 Expansion Ratio (ER)

ER values of the feed extrudate are influenced by the extent of pressure developed, which in turn is influenced by several factors like dough moisture content, die dimensions, and feed ingredient composition, residence time, and rheological behavior of the dough. Botting (1991) and Fallahi et al. (2012) indicated that high levels of temperatures and shear forces during extrusion processing change the internal structure of the dough during cooking process. Medium to high moisture content in the aqua feed mix increases the flow rate by increasing the lubricating action and reducing the pressure, resulting in less expanded structure (Pillay, 1990). Low ER values of <1.0 observed in the present extrusion studies can be due to significant influence of lipid and protein present in aqua feed mix, which might have interfered with the gelatinization process by developing protein networks with starch and also starch-lipid complexes. Mohamed (1990) in his studies on extrusion of starch-based products concluded that the ER of the extruded product is unfavorably affected by increased moisture and protein content. In the present results lower barrel temperatures favored lowering the ER values. **In their studies on the extrusion of distiller dried grains flour blends, Kim et al (1989) found that higher temperatures can result in higher ER values due to more starch gelatinization and more excretion of super-heated steam.** In the present study, lower screw speeds, lower barrel temperature, medium L/D ratio, and medium feed moisture content significantly influenced the ER which might have reduced viscosity of the feed mix and consequently reducing the ER values.

4.3 True Density (TD)

True density (TD) is influenced by screw speed, L/D ratio, and barrel temperatures based on RSM and GA analysis. In general, higher screw speeds reduce the viscosity and in turn increase the density of the product. An increase in true density coupled with an increase in screw speed can be due to the increase in non-Newtonian dough density. In the present study, a medium L/D ratio resulted in consistent TD values, which might be due to laminar flow of the feed in the metering zone. Also, an increase in screw speed might have distributed throughout the dough more evenly and might have uniformly distributed the free moisture and resulted in uniform puffing as the pellet leaves the die. The presence of lipid in the aqua feed mix might result in the formation of amylase–lipid complexes which, in turn, could have reduced the viscosity of the extrudate. According to Gehring et al. (2009), the density of the feed pellets decrease with increase in moisture content. In the present study, the low barrel temperatures and medium moisture content that were observed possibly might have decreased the extent of gelatinization and the content of the superheated steam, yielding a high density product as observed by Kim et al. (1989) and Harper (1981).

5. CONCLUSIONS

In the present study, extrusion experimental data was analyzed using response surface methodology (RSM) and genetic algorithm (GA). Regression equations developed has adequately described the effect of process variables on the quality attributes with coefficient of determination values of >0.95. RSM analysis indicated that a medium screw speed of 60-80 rpm, L/D ratio of 12-13, and feed moisture content of 30-40% and barrel temperature of $\leq 80^{\circ}\text{C}$ for ER and for TD $> 90^{\circ}\text{C}$ maximized WS, ER and TD. Based on GA analysis maximum WS of 98.10%, ER of 0.99 and TD of 1346.9 kg/m^3 was predicted at a screw speed of 96.71, 60.37 and 90.24 rpm, L/D ratio of 13.67, 12.18 and 13.52, barrel temperature of 96.26, 68.50 and 64.88°C and feed moisture content of 33.55, 33.61 and 38.36%. Based on GA analysis L/D ratio of 12-13.5, medium feed moisture content of 33-38% maximized the WS, ER and TD. **Screw speed, barrel temperature and feed moisture content are the interacting process variables, influencing maximum the WS followed by ER and TD.**

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