



## Journal of Experimental Biology and Agricultural Sciences

<http://www.jebas.org>

ISSN No. 2320 – 8694

### Ecological desalination of anchovy residues and their mixture with soybean meal for the production of poultry feed: Optimization of waste through response surface methodology (RSM)

Ilham BOUMENDIL<sup>1\*</sup> , Mhammed SISOUANE<sup>2</sup> , Youness EL HAIMER<sup>3</sup> ,  
Nabil BOUNOUAR<sup>2</sup>, Jihane KHAMLICH<sup>1</sup>, Asmae BAGGAR<sup>1</sup> , Amal SAFTI<sup>1</sup> 

<sup>1</sup>Laboratory Biochemistry Environment and Agri-food, Department of Biology, Faculty of Science and Technics Mohammedia, Hassan II University Casablanca, Morocco.

<sup>2</sup>Laboratory of Water and Environnement(LEE), Faculty of Sciences, Chouaib Doukkali University, PO Box 20, El Jadida, M-24000, Morocco.

<sup>3</sup>Laboratory of Coordination and Analytical Chemistry (LCCA), Faculty of Sciences, Chouaib Doukkali University, PO Box 20, El Jadida, M-24000, Morocco.

Received – June 13, 2023; Revision – October 06, 2023; Accepted – November 25, 2023

Available Online – November 30, 2023

DOI: [http://dx.doi.org/10.18006/2023.11\(5\).834.844](http://dx.doi.org/10.18006/2023.11(5).834.844)

#### KEYWORDS

Salted anchovy bones

Poultry feed

Soybean meal

Response surface methodology

#### ABSTRACT

Salted anchovy bones are a non-recyclable waste product containing high salt levels. However, they also contain valuable minerals such as calcium, phosphorus, potassium, magnesium, and nitrogen. This study aimed to find a cost-effective method to desalinate anchovy bones while preserving their nutritional value and repurposing them as a raw material for poultry feed. Through various tests, we were able to reduce the salt content of the anchovy bones from 15.4% to 4.7% using a 50/50 percent mixture of tap water and from 15.4% to 3.7% using a mixture of tap water and soybean meal in a 30/70 percent ratio. Combining soybean meal with desalted anchovy bones resulted in a nutritional composition comparable to that found in poultry feed, reducing salt content. The response surface method (RSM) was employed to determine the optimal proportions of desalted anchovy bones (70-90%) and soybean meal (10-30%) and to study the variables affecting the concentrations of NaCl, Ca, P, Ash, and TNM. The study revealed the influence of desalted anchovy bone and soybean meal percentages on these concentrations. This study demonstrates that the method used provides an ideal approach for understanding the interactions between input parameters (% DAR, % SM) and output parameters (NaCl, Ca, P, Ash, and TNM) and shows promising results for the desalination of anchovy bones using a soybean meal cake as well as the feasibility of creating poultry feed.

\* Corresponding author

E-mail: [ilhamboumendil@gmail.com](mailto:ilhamboumendil@gmail.com) (Ilham BOUMENDIL)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

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## 1 Introduction

The European anchovy (*Engraulis encrasicolus*) is a highly significant small pelagic fish in terms of biomass and commercial interest (Fernández-Corredor et al. 2021), and it represents one of the most important fisheries in the Mediterranean region (Paone et al. 2021). The production of anchovy fillets generates substantial biological waste in the form of bones (Paone et al. 2021). Maritime countries eat primarily fish (Nurulnadia et al. 2021). Moreover, salting anchovies results in a large volume of residues containing a high NaCl content, which is typically discarded (Marchetti et al. 2021). These discards are considered wasteful regarding fishery resources, representing a significant portion of the overall marine harvest (Washington and Ababouch 2011, Ababouch et al. 2009). Fish bones comprise approximately 70% inorganic and 30% organic matter by weight (Kizilkaya et al. 2010). The majority of the inorganic fraction is composed of hydroxyapatite (HAP), which is a form of calcium phosphate with the chemical formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  (Kizilkaya et al. 2010). Salt waste poses a significant challenge to the recycling of fishery products (Boumendil et al. 2020). Unfortunately, information regarding salted fishmeal is scarce (Hasan et al. 2019). Given its properties, the biological waste from canned fish, specifically the bones, can serve as a starting point for poultry feed production after being rinsed with drinking water to reduce its high NaCl content. Plant by-products are considered nutrient-rich sources and inexpensive functional substances with diverse potential applications (Taarji et al. 2018). Soy sauce is an excellent alternative to replace NaCl without compromising the nutritional value of the feed (Kremer et al. 2009). However, soybean meal is highly sought-after in the animal feed sector due to its protein richness. Therefore, in this study, the desalted waste derived from anchovy bones will be valorized by incorporating soybean meal as a co-substrate. The primary objective of this study is to develop an environmentally friendly desalination process that preserves the nutritional value of anchovy bones. The secondary aim is to enhance the value of these bones in the animal feed sector. To achieve these goals, the response surface method (RSM) will be employed to determine the optimal ratios of desalted anchovy bone waste (70-90%) and

soybean meal (10-30%) for desalination anchovy bones and preparing poultry feed.

## 2 Materials and Methods

### 2.1 Raw materials

In this study, salted anchovy (*Engraulis encrasicolus*) bones were collected in August 2022 from an industrial plant in Morocco. They were then transported in a cooling box at approximately 4°C.

### 2.2 Preparation of Anchovy Bones

Under laboratory conditions, the salted anchovy bones were separated from the meat and dried at 60°C until a constant weight was achieved. The dried bones were ground by a blender and sieved to a size of 800 µm for further processing.

### 2.3 Removal of Salt Content

A solid-liquid extraction method was employed to remove the salt content from the anchovy bones. The bones were immersed in potable water in a ratio of 50% bones to 50% water (m/v). The extraction process was conducted at 25°C with magnetic stirring at 300 rpm for 24 hours. After the extraction, the mixture was filtered, and the resulting solution was stored in a refrigerator until its final use.

### 2.4 Physicochemical analysis of anchovy bones and soybean meal

The results of each desalination test represent the average of three repetitions. The NaCl salt content in anchovy bones was determined using the titrimetric method (Ababouch et al. 2009). The ash content was obtained by incinerating the sample for 12 hours at a temperature of 550°C (Köprücü and Özdemir 2005). The resulting ash was dried at 105°C until a constant mass (AOAC 1990). The fat content was determined at 100°C according to the procedure described in (AOAC 2005). The total nitrogen content was determined using the Kjeldahl (Horwitz and International 2000). The crude cellulose fraction was determined by the AOAC



Figure 1 The raw materials used in this study: (a) salted anchovy bones, (b) desalted anchovy bones and (c) soybean meal

method (AOAC and Horwitz 1975). The Ca, Mg, K, and P ions concentrations were measured following the protocols described in AOAC and Horwitz (1975).

## 2.5 Experimental Design

To analyze the collected data and determine the correlation between the dependent and independent variables, regression analysis with Response Surface Methodology (RSM) was employed using Design-Expert software version 13.0.14.0. The objective was to investigate the response (Yi) of specific constant factors, i.e., Salt (%), Ca (g/kg), P (g/kg), ash (%), and TNM (%). A mathematical equation representing each response surface (Yi) was used. The main purpose of RSM is to optimize operating conditions that meet specific requirements (Myers et al. 2016).

The polynomial equation (1), shown at the bottom of the diagram, describes the response Y:

$$Y = f(y) = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \varepsilon \quad (1)$$

In equation (1), Yi represents the predicted response corresponding to the independent variables Xi (i = 1, 2),  $\beta_0$  is the constant term,  $\beta_{ij}$  (i = 1, 2 and j = 1, 2) represents the interaction terms between variables and quadratic terms, where i and j are the coefficients of the linear terms, and  $\varepsilon$  represents the residual error.

The significance and fit of each finding in the developed model were carefully examined. Non-significant terms were removed using the same program, and an intervention surface plot was generated. The response surface method (RSM) was utilized to optimize the method parameters for modifying the nutrient composition of the manufactured poultry feed. The independent variables analyzed were the amounts of desalted anchovies and soybean meal. The goal was to maximize the yield (Y) of salts, phosphorus (g/Kg), TNM%, Ash%, and Ca (g/Kg). The central Composite Design (CCD) is a useful technique in statistical design that allows the investigation of component impacts on responses and optimization studies. By employing this technique, a quadratic surface can be fitted, enabling the analysis of parameter relationships while reducing the number of required tests. A CCD-based batch experiment was conducted to evaluate the effect of desalted anchovy and soybean meal proportions on poultry feed synthesis. The design space was constructed in the initial tests, and

the factors were encoded at  $\pm 1$  for the factorial points and 0 for the center point. As shown in Table 1, these codes indicate the range of variation for each factor. This study aimed to develop a method for natural desalination by combining soybean meal and desalted anchovies to create an optimal poultry feed.

## 3 Results and Discussion

### 3.1 Nutrient composition of raw materials

Unused salted fishmeal, particularly in the case of salted fish, causes significant losses to the canning industries. Desalting has been investigated as a solution to this problem. It involves the solid-liquid extraction of various components, such as  $\text{Na}^+$  and  $\text{Cl}^-$  ions, and soluble proteins from the salted product into the desalting water. Research has shown that replacing salt with soy sauce is an effective method for reducing the sodium content. Consequently, the concept of desalting fishmeal using potable water and soybean meal and creating future "poultry products" was developed. The chemical composition of soybean meal (SM), desalted anchovy residue (DAR), and salted anchovy residue (SAR) produced is presented in Table 2. The salted anchovy residues were successfully rinsed to remove a significant amount of salts (from 15.4% to 4.7%) without significantly altering the other chemical characteristics of the anchovy residues.

The results presented in Table 1 are based on a comprehensive examination of the experimental parameters and independent variables. The results obtained from the various formulations tested during the experiment are shown in Table 2. The study aimed to develop a regression model that accurately represents the relationships between the dependent and independent variables. To achieve this goal, the researchers employed Response Surface Methodology (RSM) and conducted preliminary tests to optimize the process. Table 3 was used for a precise and reliable evaluation of the variable responses before constructing the RSM mathematical model and determining the ideal experimental conditions to achieve the desired response values. The study focused on analyzing the effects of desalted anchovy proportions (X1), soybean meal proportions (X2), and their interactions on the levels of salt (%), Ca (g/Kg), P (g/Kg), Ash (%), and TNM (%) at two distinct levels.

The data presented in Table 3 were obtained from empirical assessments, as described in Table 4, and served as the basis for

Table 1 Factor levels of independent variables for poultry feed synthesis.

Independent variables	Variables coded	Unit	Range and level of actual and coded values		
			-1	0	1
Desalted Anchovies Residues (DAR) (%)	X1	%	70	80	90
Soybean Meal (SM) (%)	X2	%	10	20	30

Table 2 Chemical composition of anchovy bones and soybean meal

Settings	Samples		
	SAR	DAR	SM
%Salt	15.4	4.70	0.4
Dry matter %	94.83	96.18	90.40
Ash%	16.09	13.98	7.50
Ca (g/Kg)	34.80	27.15	4.2
Mg (g/Kg)	3.50	3.02	3.0
K (g/Kg)	3.80	3.10	22.1
P(g/Kg)	17.4	14.2	6.8
TNM %	34.12	29.73	38.08
MG%	7.12	5.83	3.31
CB%	0.00	0.00	6.39

SAR: salted anchovies residues, DAR: desalted anchovies residues, and SM: soybean meal.

Table 3 Real value with responses of independent factors

Factors			Responses			
DAR (%)	SM (%)	Salt (%)	Ca (g/Kg)	P (g/Kg)	Ash (%)	TNM (%)
70	10	3.33	19.425	10.62	10.536	24.619
70	20	3.37	19.845	11.3	11.286	28.427
70	30	3.41	20.265	11.98	12.036	32.235
80	10	3.8	22.14	12.04	11.934	27.592
80	20	3.84	22.56	12.62	12.84	31.34
80	20	3.94	22.5	12.72	12.684	31.4
80	20	3.89	22.6	12.82	12.64	31.44
80	20	3.78	22.66	12.66	12.74	31.49
80	30	3.88	22.98	13.4	13.434	35.208
90	10	4.27	24.855	13.46	13.332	30.565
90	20	4.31	25.275	14.14	14.082	34.373
90	30	4.35	25.695	14.82	14.832	38.181

SAR: salted anchovies residues, DAR: desalted anchovies Residues, SM: soybean meal

the models created in this study. These models established the relationship between the independent variables and the five response variables are salt (%), Ca (g/Kg), P (g/Kg), Ash (%), and TNM (%), using a second-order polynomial equation. Desalted anchovy and soybean meal amounts were denoted as X1 and X2, respectively. The two-factor interaction coefficients (X1X2) and the second-order terms (X1<sup>2</sup> and X2<sup>2</sup>) represented the quadratic impact and the interaction between two factors, respectively. The coefficients (X1 and X2) with only one factor represented the separate effects of each component. According to Filli et al.

(2010), favourable regression terms indicated synergistic effects, while negative values indicated antagonistic effects.

### 3.2 Analysis of variance (ANOVA)

Analysis of variance (ANOVA) was used to analyze the principal composite design, dividing the information into four distinct categories, i.e. sequential P-value, unadjusted P-value, adjusted R-squared value, and predicted R-squared value. The P-value summary of the ANOVA study (Table 3) showed a link with the

Table 4 Assessment of the second-order polynomial equation derived for the five responses

Response variables	Second-order polynomial models	Regression coefficient	
		R <sup>2</sup>	Radj <sup>2</sup>
SALT %	$-0.75+0.065*\text{DAR}+0.0085*\text{SM}+5.86\text{E}-13*\text{DAR} * \text{SM}-1.112\text{E}-4\text{DAR}^2-1.12\text{E}-4*\text{SM}^2$	0.9893	0.9804
TNM%	$-0.58+0.311*\text{DAR}+0.3843*\text{SM}+2.93\text{E}-12\text{DAR} * \text{SM}- 8.7\text{E}-5*\text{DAR}^2-8.7\text{E}-5*\text{SM}^2$	0.9999	0.9998
Ash%	$-1.39+0.083\text{SM}+ 3.58\text{E}-12*\text{DAR} * \text{SM}-2.1\text{E}-4*\text{DAR}^2-2.1\text{E}-4*\text{SM}^2$	0.9984	0.9971
Ca( g/Kg)	$-0.66+0.288\text{DAR}*\text{DAR}+0.046*\text{SM}+6.2145\text{E}-17\text{DAR}*\text{SM}-0.1\text{E}-3*\text{DAR}^2-0.1\text{E}-3*\text{SM}^2$	0.9997	0.9994
P(g/Kg)	$0.50+0.13\text{DAR}*\text{DAR}+0.065*\text{SM} +4.15637\text{E}-17 * \text{DAR}*\text{SM}+7.5\text{E}-5*\text{DAR}^2 -7.5\text{E}-5*\text{SM}^2$	0.9985	0.9972

\*TNM: Total Nitrogen Material; \*DAR: Desalted Anchovies Residues; \*SM: Soybean Meal; \*R<sup>2</sup>: coefficient of determination\* Radj: Adjusted R<sup>2</sup>.

proposed quadratic model. Any words with a P-value equal to or higher than 0.05 are now considered significant phrases according to the 95% criterion used.

ANOVA F-value tests were employed to assess the significance of each model type. In addition, a goodness-of-fit test was conducted to evaluate the appropriateness of the models. This test determines the suitability of the provided model by using it as the numerator in an F-test to examine the null hypothesis. The lack of fit was identified by comparing the variance around the model with the inherent variation within the repeated data. This test, as per the approach of Bhatti et al. (2011), has been employed for estimating model diversity. It was subsequently argued that the least significant quadratic model may not be suitable.

The results indicate that the linear and quadratic terms were the main variables influencing the performance of the parameter under

study. The number of terms in the model's R-squared was used to assess its ability to explain variability around the mean. However, Taran and Aghaie (2015) found that the adjusted R-squared tends to decrease as the number of terms increases, suggesting that adding more terms does not necessarily improve the model's value. The statistical program used in this analysis also tested regression models and assessed the significance of model-specific coefficients without considering a goodness-of-fit test. The significance of each coefficient was determined using the P-value, highlighting the importance of understanding the inherent interactions among the tested components (Shrivastava et al. 2008). As stated, a higher F-test result and a lower P-value indicate a greater significance level for the corresponding coefficient. The coefficient of variation (CV), obtained by dividing the estimate's standard deviation by the mean value of the observed response, was employed to assess the replicability and reproducibility of the models (Shrivastava et al. 2008). The calculations yielded CV values of 0.14% for TNM,

Table 5 Analysis of variance (ANOVA) for the full quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
TNM Model	140.05	5	28.01	13685.44	< 0.0001	Significant
X <sub>1</sub> -DAR	53.03	1	53.03	25913.34	< 0.0001	
X <sub>2</sub> -SM	87.01	1	87.01	42513.56	< 0.0001	
X <sub>1</sub> X <sub>2</sub>	0.0000	1	0.0000	0.0000	1.0000	
X <sub>1</sub> <sup>2</sup>	0.0002	1	0.0002	0.0998	0.7628	
X <sub>2</sub> <sup>2</sup>	0.0002	1	0.0002	0.0998	0.7628	
Residual	0.0123	6	0.0020			
Lack of Fit	0.0002	3	0.0001	0.0169	0.9964	Not significant
Pure Error	0.0121	3	0.0040			
Cor Total	140.05	11				
Std. Dev.	0.0452	R <sup>2</sup>		0.9999		
Mean	31.41	Adjusted R <sup>2</sup>		0.9998		
CV %	0.1440	Predicted R <sup>2</sup>		0.9999		
		Adeq Precision		423.9649		

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Salt Model	1.34	5	0.2672	111.24	< 0.0001	Significant
X <sub>1</sub> -DAR	1.33	1	1.33	551.77	< 0.0001	
X <sub>2</sub> -SM	0.0096	1	0.0096	4.00	0.0925	
X <sub>1</sub> X <sub>2</sub>	0.0000	1	0.0000	0.0000	1.0000	
X <sub>1</sub> <sup>2</sup>	0.0003	1	0.0003	0.1405	0.7207	
X <sub>2</sub> <sup>2</sup>	0.0003	1	0.0003	0.1405	0.7207	
Residual	0.0144	6	0.0024			
Lack of Fit	0.0003	3	0.0001	0.0240	0.9940	Not significant
Pure Error	0.0141	3	0.0047			
Cor Total	1.35	11				
Std. Dev.	0.0490	R <sup>2</sup>	0.9893			
Mean	3.85	Adjusted R <sup>2</sup>	0.9804			
CV %	1.27	Predicted R <sup>2</sup>	0.9817			
		Adeq Precision	29.4321			
Ash Model	15.10	5	3.02	770.40	< 0.0001	Significant
X <sub>1</sub> -DAR	11.73	1	11.73	2990.42	< 0.0001	
X <sub>2</sub> -SM	3.37	1	3.37	860.68	< 0.0001	
X <sub>1</sub> X <sub>2</sub>	0.0000	1	0.0000	0.0000	1.0000	
X <sub>1</sub> <sup>2</sup>	0.0012	1	0.0012	0.2999	0.6037	
X <sub>2</sub> <sup>2</sup>	0.0012	1	0.0012	0.2999	0.6037	
Residual	0.0235	6	0.0039			
Lack of Fit	0.0012	3	0.0004	0.0526	0.9813	not significant
Pure Error	0.0224	3	0.0075			
Cor Total	15.13	11				
Std. Dev.	0.0626	R <sup>2</sup>	0.9984			
Mean	12.70	Adjusted R <sup>2</sup>	0.9971			
CV %	0.4932	Predicted R <sup>2</sup>	0.9971			
		Adeq Precision	97.0203			
Ca Model	45.29	5	9.06	3919.03	< 0.0001	Significant
X <sub>1</sub> -DAR	44.23	1	44.23	19136.83	< 0.0001	
X <sub>2</sub> -SM	1.06	1	1.06	457.96	< 0.0001	
X <sub>1</sub> X <sub>2</sub>	0.0000	1	0.0000	0.0000	1.0000	
X <sub>1</sub> <sup>2</sup>	0.0003	1	0.0003	0.1154	0.7457	
X <sub>2</sub> <sup>2</sup>	0.0003	1	0.0003	0.1154	0.7457	
Residual	0.0139	6	0.0023			
Lack of Fit	0.0003	3	0.0001	0.0196	0.9955	not significant

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Pure Error	0.0136	3	0.0045			
Cor Total	45.30	11				
Std. Dev.	0.0481	R <sup>2</sup>	0.9997			
Mean	22.57	Adjusted R <sup>2</sup>	0.9994			
CV %	0.2130	Predicted R <sup>2</sup>	0.9995			
		Adeq Precision	184.4472			
P Model	14.87	5	2.97	781.09	< 0.0001	Significant
X <sub>1</sub> -DAR	12.10	1	12.10	3176.82	< 0.0001	
X <sub>2</sub> -SM	2.77	1	2.77	728.51	< 0.0001	
X <sub>1</sub> X <sub>2</sub>	0.0000	1	0.0000	0.0000	1.0000	
X <sub>1</sub> <sup>2</sup>	0.0001	1	0.0001	0.0394	0.8492	
X <sub>2</sub> <sup>2</sup>	0.0001	1	0.0001	0.0394	0.8492	
Residual	0.0229	6	0.0038			
Lack of Fit	0.0001	3	0.0000	0.0066	0.9991	not significant
Pure Error	0.0227	3	0.0076			
Cor Total	14.90	11				
Std. Dev.	0.0617	R <sup>2</sup>	0.9985			
Mean	12.72	Adjusted R <sup>2</sup>	0.9972			
CV %	0.4853	Predicted R <sup>2</sup>	0.9975			
		Adeq Precision	96.2491			

Table 6 Responses of synthetic poultry feeds to predicted values.

Run Order	SALT		Ca		P		TNM		Ash	
	Actual Value	Predicted Value	Actual Value	Predicted Value	Actual Value	Predicted Value	Actual Value	Predicted Value	Actual Value	Predicted Value
1	3.84	3.86	22.56	22.58	12.62	12.71	31.34	31.41	12.84	12.72
2	3.37	3.38	19.84	19.85	11.30	11.30	28.43	28.43	11.29	11.30
3	3.41	3.41	20.27	20.26	11.98	11.98	32.23	32.23	12.04	12.03
4	3.80	3.81	22.14	22.15	12.04	12.04	27.59	27.60	11.93	11.95
5	4.35	4.35	25.70	25.69	14.82	14.82	38.18	38.18	14.83	14.83
6	3.88	3.89	22.98	22.99	13.40	13.40	35.21	35.21	13.43	13.45
7	4.31	4.32	25.27	25.28	14.14	14.14	34.37	34.38	14.08	14.10
8	4.27	4.27	24.86	24.85	13.46	13.46	30.57	30.56	13.33	13.33
9	3.33	3.33	19.43	19.42	10.62	10.62	24.62	24.62	10.54	10.53
10	3.94	3.86	22.50	22.58	12.72	12.71	31.40	31.41	12.68	12.72
11	3.89	3.86	22.60	22.58	12.82	12.71	31.44	31.41	12.64	12.72
12	3.78	3.86	22.66	22.58	12.66	12.71	31.49	31.41	12.74	12.72

1.27% for salt, 0.49% for ash, 0.21 g/Kg for Ca, and 0.49 g/Kg for P, indicating that the model had reasonable reproducibility. The independent variables were used to generate projected values for the model, and a comparison with the experimental outcomes

revealed a strong correlation between the actual experimental response and the predicted response (Table 6). These results demonstrate the effectiveness of the approach for synthesizing poultry feed.

### 3.3 Checking Model Suitability

The models constructed using RSM must provide an accurate approximation to ensure a reliable assessment of the actual environment (Table 4). Filli et al. (2010) suggested that the verification procedure primarily employs the graphical and numerical approaches. The graphical approach considers the type of residuals representing the discrepancy between observed and

predicted values within the model, while the numerical method assesses the coefficient of determination ( $R^2$ ) and adjusted  $R^2$  ( $Radj^2$ ).

When assessing the appropriateness of a model, the residuals of least-squares adjustments play a crucial role (Myers et al. 2004). The distribution of predicted values versus actual experimental values for SALT, Ca, P, TNM, and Ash is presented in Figure 2 (a,

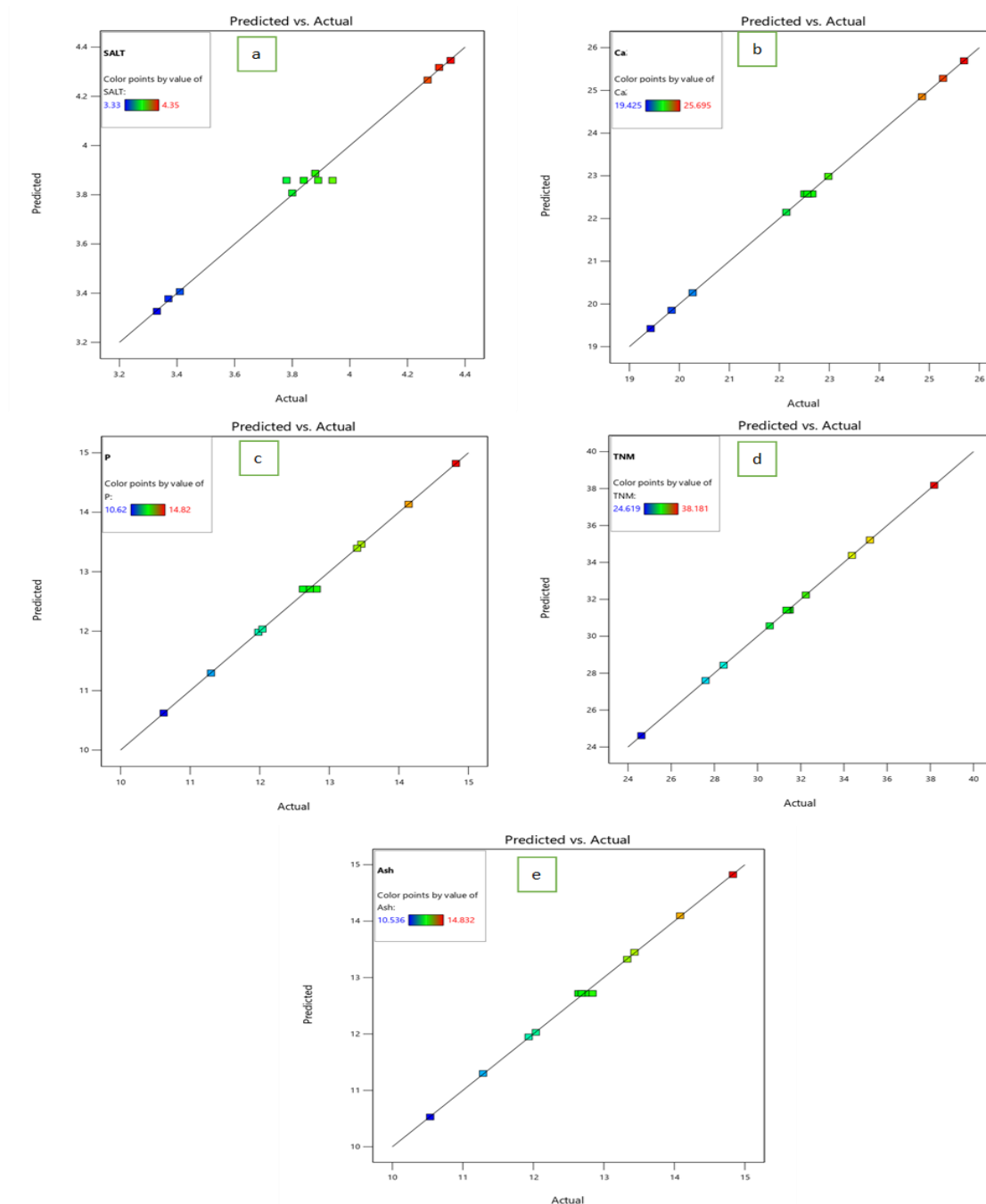


Figure 2 a, b, c, d and e displayed different graphs depicting predicted values versus experimental values for SALT, Ca, P, TNM, and Ash



b, c, d, and e). Regression coefficients of 98.93%, 99.97%, 99.85%, 99.99%, and 99.84% are determined by comparing each observed value with the predicted values obtained from the models, confirming that the regression model accurately describes the experimental data. The presence of evenly distributed points in the plots, forming a linear pattern, further supports the validity of the models. This alignment of points validates the assumption of typicality made in the study. In this context, 'n' represents the number of experimental trials, while 'p' denotes the number of predictors in the model, excluding the constant term. According to

Koocheki et al. (2009), an ideal  $R^2$  should exceed 80%, while Chauhan and Gupta (2004) suggested an  $R^2$  more significant than 78% is acceptable. The models in this study exhibit  $R^2$  values ranging from 99.84% to 99.99% and  $R^2_{adj}$  values ranging from 98.04% to 99.98%, with both  $R^2$  and  $R^2_{adj}$  values approaching 1 (Lee and Wang 1997; Zaibunnisa et al. 2009). These values are obtained by combining the two independent components mathematically. The significance of  $R^2$  approaching unity is highlighted by the widely accepted notion that a higher  $R^2$  indicates a better fit of the model to the experimental data. The

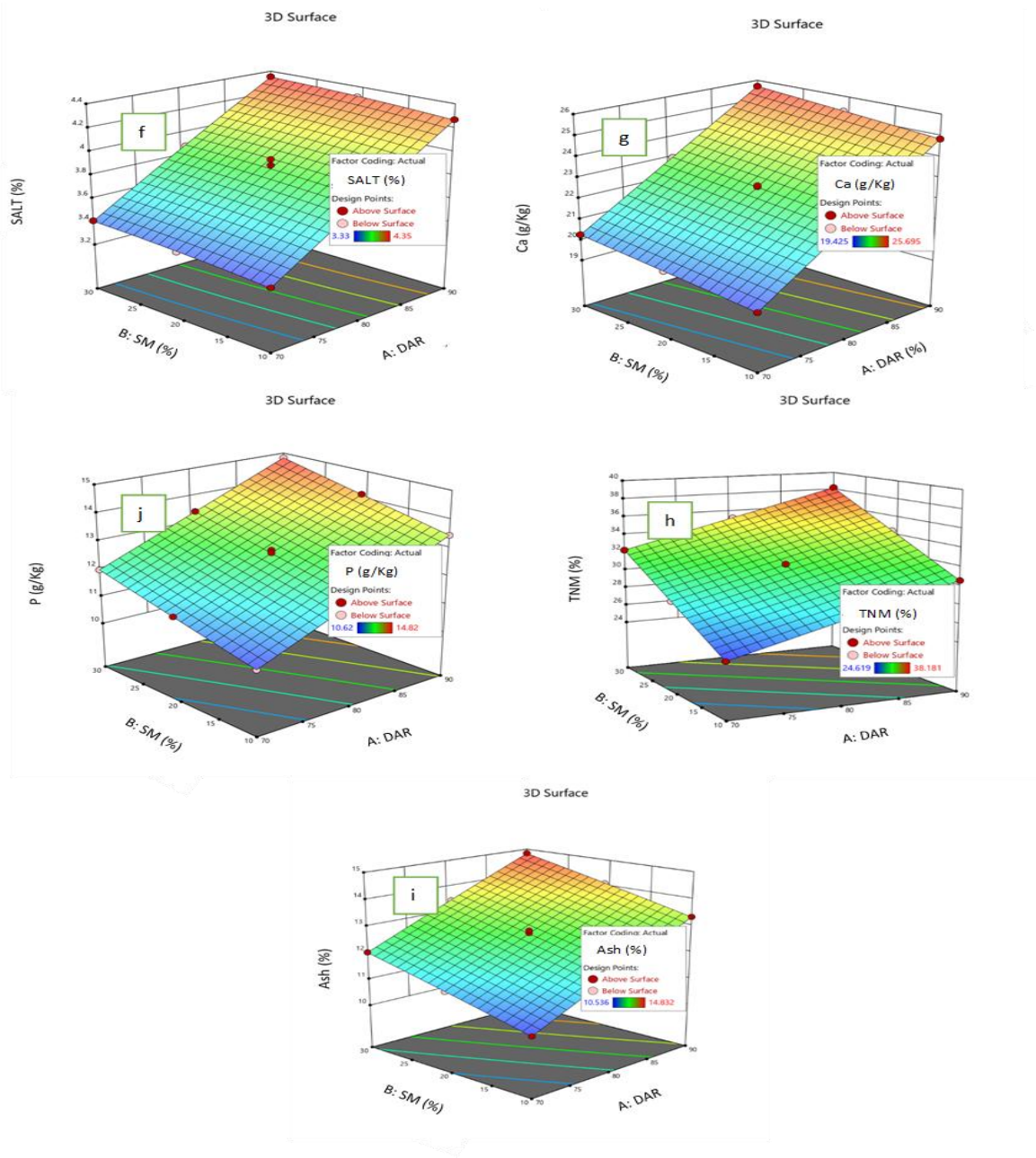


Figure 3 The interaction 3D of % SM and % DAR (f, g, h, i and j)

idea that adding additional components to a model continuously improves  $R^2$ , regardless of their statistical significance, is no longer considered novel. Therefore, Koocheki et al. (2009) suggested that an  $R^2_{adj}$  exceeding 90% is a more acceptable criterion for determining model suitability, as a high  $R^2$  does not always guarantee an adequate model. The higher  $R^2$  values obtained in this experiment suggest the absence of non-essential terms in the model. In conclusion, the overall analysis from Figure 2 suggests that the distribution of poultry feed appears random and visually pleasing. This indicates that the model consistently captures the variability across all response values (Y). Thus, it is reasonable to infer that the model accurately represents the formulation of an organic anchovy residue and soybean meal-based poultry feed additive. The visual appearance of the plots in Figure 2 further supports this conclusion.

### 3.4 Interactive factors and effects on the addition of soybean meal for desalination

The response surface plots generated by the models visually represent the complex relationship between the independent and dependent variables. These three-dimensional graphs illustrate the intricate connections. The dataset was analyzed using the linear, quadratic, and interaction terms specified in Table 4 to create these visualizations. Liu et al. (2009) explained that each contour line on the graph represents a specific elevation above the plane, indicating the overlapping levels of the independent variables. The 3D response surfaces were constructed by carefully examining the variations in the two variables and observing how each response changed across the experimental range. Table 5 demonstrates a statistically significant interaction between % SM and % DAR, as indicated by the P-values ( $P = 1.0000$  for MTN, salt, ash, and Ca). Notably, a higher % DAR led to higher TNM and ash percentages, while higher SM and DAR led to higher P and Ca percentages. Conversely, the percentage of salt decreases from 4.35% to 3.33% with soybean meal. This behaviour is illustrated in Figure 3, with preliminary validation shown in Table 3. The results were largely consistent with the predictions made by the model. The remarkable agreement between the confirmed results and the projected or anticipated yields of Ca, P, TNM, Ash, and salt, as shown in Table 6, ensures the accuracy of the models. Additionally, the reasonably high  $R^2$  values (99.97% for Ca, 99.85% for P, 99.99% for TNM, 99.84% for Ash, and 98.93% for salt) demonstrate a strong resemblance between the lightly evaluated experimental values and the anticipated or projected values reported in Table 6. This further supports the accuracy and reliability of the suggested model.

### 3.5 Process optimization and validation of the waste associations

The model developed for process parameters underwent simplification using the response optimization program integrated into Design-Expert version 13.0.14.0. This software provides

optimal solutions regardless of the combinations of input variables. The highly interactive optimization approach considers the trade-offs across various independent components and responses (Agu et al. 2015). The response surface method (RSM) has proven effective in illustrating the relationship between response variables, particularly the percentage yield, and the variables involved in the desalination process. The reactions of interest include Salt, TNM, Ash, P, and Ca, while the process parameters encompass DAR and SM. The response optimizer effectively utilizes the generated models to generate the best outcomes for both the response variables and the independent factors.

### Conclusion

The response surface method (RSM) was employed to determine the optimal parameters for the desalination of organic waste treatment and the synthesis of poultry feed. A second-degree polynomial model was utilized, and this approach proved effective in defining and predicting process responses to variations in input variables within the experimental range. The validity of the models was confirmed by fitting factor estimates to the model equations and obtaining consistent results. The optimization process, which included the amount of soybean meal and the main minerals TNM, Ash, Ca, P, and salt, allowed for the identification of the optimal conditions for desalting anchovy bones. The characteristics of soybean meal, in particular, highlight its commercial feasibility as a product for the desalination anchovy bones and its potential use as a future poultry feed.

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