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Optimisation of gelatine extraction from chicken feet-heads blend using Taguchi design and response surface methodology

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Abstract

The present work investigated the optimisation of gelatine extraction yield with interesting techno-functional properties from chicken heads-feet by-product blend. Taguchi L27 orthogonal experimental design was used to optimise the extraction parameters, including acetic acid concentration (2, 3.5, and 5%), extraction temperature (55, 65, and 75°C), and extraction time (2, 4, and 6 h), with yield, viscosity, emulsifying activity index (EAI), and foaming capacity (%) as responses. The collected data were modelled and optimised using the response surface method (RSM) and desirability function (DF). Based on the data obtained, the optimal extraction parameters were an acid concentration of 3.06% and an extraction temperature of 75°C for 6 h. Responses to these extraction conditions included a yield of 10.97%, an EAI of 24.22 m^2/g, a viscosity of 3.36 mPa.s, and a foaming capacity of 45.07%. Under these ideal conditions, the verified and predicted values were found to be almost identical. As a result, the estimate models are trustworthy and safe for predicting the dependent variables. The findings indicated that a blend of chicken feet and heads could be a source of gelatine with interesting functional properties.

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Introduction

Transforming live animal into carcass for human use yields novel products mostly constituted of muscular tissue (meat) and other organic by-products (Seidavi *et al*., 2019). These by-products are protein-rich, especially collagen from which gelatine is extracted. Gelatine is a natural hydrocolloid widely used as emulsifying, foaming, texturing, and gelling ingredients in various food preparation (Gimenez *et al*., 2005), such as meat (Schrieber and Gareis, 2007), dairy products (Arioui *et al*., 2017), and marshmallow (Mardani *et al*., 2019). In addition, gelatine is widely utilised in the biomedical, pharmaceutical, and cosmetic industries (Schrieber and Gareis, 2007). This biopolymer is a soluble protein derived from the partial heat hydrolysis of collagen (Sarbon *et al*., 2013).

Gelatine is mostly derived from mammalian by-products, primarily porcine skin, bovine hides, and bones, which account for 41, 28.5, and 29.5% of commercial gelatine sources, respectively (Milovanovic and Hayes, 2018). However, mammalian gelatine causes various socio-cultural, health, and religious concerns. As a result, obtaining gelatine from various by-product sources is gaining popularity (Karim and Bhat, 2009). Waste from poultry slaughterhouses and chicken processing companies is a suitable raw material for collagen extraction (Zain *et al*., 2019), and thus, gelatine.

The poultry industry continues to expand and industrialise in many regions of the world, with worldwide chicken meat output increasing from 9 to 133 million tons between 1961 and 2020, accounting for about 40% of total meat production (UNFAO, 2022). With increased chicken consumption, the

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poultry industry generates more waste and by-products which must be managed for environmental and economic reasons. Chicken feet and heads are two of the most common chicken slaughterhouse by-products that could be used to create a value-added product. They contain little bones, and high amount of cartilage (Chakka *et al*., 2017; Ee *et al*., 2019) which makes them a good source of collagen and gelatine (Erge and Zorba, 2018; Mokrejš *et al*., 2019; Gál *et al*., 2020; Ab Rahim *et al*., 2021).

Most studies have used chicken feet (Araújo *et al*., 2018; Mokrejš *et al*., 2019) or heads (Ee *et al*., 2019; 2021). However, no study has used the mixture of feet and heads as raw materials to extract the gelatine. The present work used Taguchi design and response surface methodology (RSM) to optimise the extraction parameters, such as acetic acid concentration, hydrolysis time, and temperature, thus resulting in a higher gelatine extraction yield from chicken feet and head mixtures with the best techno-functional properties.

Materials and methods

Raw material

Roughly 15 kg of fresh chicken feet and heads were obtained from the slaughter and meat processing factory of the Poultry Group West Algeria Mostaganem (GAO-ORAVIO) in western Algeria. The samples were brought to the laboratory, and rinsed with tap water (de-nailed and plucked). The feet and heads were then sliced into small (5 cm) pieces, and stored in plastic bags at -20°C in equal parts (50 g feet pieces + 50 g heads pieces) until gelatine extraction. Sigma-Aldrich, a chemical manufacturer business, provided food-grade acetic acid. All of the reagents and chemicals used were of the highest grade.

Proximate composition of raw material

The raw material's moisture, ash, and crude fat contents were determined using the method prescribed by the Association of Official Analytical Chemists (AOAC, 1995). The moisture content was determined gravimetrically after oven-drying the sample at 105°C to a constant mass. The ash content was determined after incineration of the sample at 550°C in a muffle furnace. The crude fats were determined by Soxhlet extraction. The protein content was determined using the Kjeldahl method (AOAC, 2000) by determining the total nitrogen content using an automatic nitrogen analysis (Gerhardt Analytical System, Gerhardt GmbH & Co. KG, Germany). The crude protein content was calculated by multiplying the determined nitrogen content by a factor of 6.25.

Modelling and optimisation of gelatine extraction

The response surface methodology (RSM) was used to optimise the experiment. The Taguchi method was used to optimise the gelatine extraction conditions from the by-product blend of feet and heads. Three independent variables (Xn), extraction temperature (°C), extraction time (h), and acetic acid concentration (%) were selected with three levels. An L27 (3^3) orthogonal matrix yielded 27 experimental runs. Four dependent parameters (Y), namely yield (%), viscosity (mPa.s), emulsifying activity index (m^2/g), and foaming capacity (%) were measured after each extraction. A regression model was established using a second-order model for each response as described in Eq. 1:

$$Y = a_0 + \sum_{i=1}^{k} a_i X_i + \sum_{\substack{i=1 \\ i \neq j}}^{k} a_{ij} X_i X_j + \sum_{i=1}^{k} a_{ii} X_i^2 + \varepsilon \quad \text{(Eq. 1)}$$

where, a_0, a_i, a_{ii}, and a_{ij} = the constant terms, the coefficients of the linear, quadratic, and interactive terms, respectively, X_i and X_j = the independent variables. ε = statistical experimental error, K = number of variables (in this case, K = 3), Y = studied response.

Design-Expert 10 software was used to create the regression models. The coefficient of determination R^2 was used to assess the polynomial model's goodness of fit. RSM validated the optimal conditions for maximum yield values, viscosity, foaming, and emulsifying capacity.

Gelatine extraction

Gelatine was extracted from the feet and heads mixture using acetic acid as described by Chakka *et al*. (2017) with modifications. Briefly, 100 g of thawed feet-heads mixture sample were soaked in 0.5 M NaOH solution in the 1/10 (w/v) ratio under stirring at room temperature for 18 h to remove non-collagen material. Subsequently, the samples were filtered and washed several times with distilled water to neutralise the pH. After filtration, the residue was treated with acetic acid at different concentrations (2, 3.5, and 5%). After 18 h of acetic acid treatment under constant stirring at 4°C, the samples were filtered

under vacuum, and the gelatine extraction was carried out in warm water with different temperatures (55, 65, and 75°C) for 2, 4, or 6 h. The extract was stirred with activated charcoal (4 g) for 20 min (Saenmuang *et al*., 2020) to remove odours and impurities. Finally, the extract was filtered, poured into Petri dishes, and dried in a vacuum oven (45°C) for 48 h. The gelatine sheets were then powdered using the electric grinder. The yield of each extraction was determined using Eq. 2:

$$Yield(\%) = (Gelatin\ dry\ weight/raw\ material\ weight) \times 100 \quad \text{(Eq. 2)}$$

Analysis

Viscosity

The dynamic viscosity of gelatine solutions was determined following the standard technique of the Gelatine Manufacturers Institute of America (GMIA, 2019) using a viscometer (HAAKE Falling Ball Viscometer Type C, Thermo Fisher Scientific, Germany). A gelatine solution (6.67%, w/v) was poured into the viscosimeter tube maintained at a constant temperature (60°C), and then a ball with a known diameter was dropped on top of the gelatine solution. A timer was used to determine the falling time of the ball as it moved from ring A to ring B. Each gelatine solution's dynamic viscosity was calculated using Eq. 3:

$$\eta = k(d1 - d2).t \quad \text{(Eq. 3)}$$

where, η = viscosity (mPa.s), K = ball constant (mPa.s.cm3/g. s), d_1 = density of the ball in g/cm3, d_2 = density of the gelatine solution (6.67%) at 60°C (d_2 = 1.003 g/cm^3), and t = time (seconds).

Emulsification activity

Emulsification activity (EAI) was determined according to Pearce and Kinsella (2002). Briefly, 10 mL of sunflower oil were homogenised with 30 mL of 1% gelatine solution for 1 min. After homogenisation, 50 µL aliquots of each emulsion were added to 5 mL of 0.1% SDS solution. The absorbance was measured at 500 nm after vigorous vortexing. The emulsifying activity was calculated using Eq. 4:

$$EAI\ (m2/g) = \frac{2 \times 2.303 \times A0 \times 100}{0.01 \times 0.25 \times C} \quad \text{(Eq. 4)}$$

where, A0 = emulsification absorbance at 0 min, and C = gelatine weight per unit volume (g/m^3).

Foaming property

The foaming property was determined using the Sathe and Salunkhe (1981) method. Briefly, 20 mL of 1% gelatine solution were homogenised at room temperature for 2 min using a homogeniser (wise Tis® HG-15A). The whipped sample was immediately transferred to a 100 mL graduated cylinder, and the volume Vt was noted. The foaming capacity was calculated using Eq. 5:

$$FE(\%) = ((Vt - V0) \times 100)/V0 \quad \text{(Eq. 5)}$$

where, Vt = total volume of the homogenised solution after 10 min of standing at room temperature, and V0 = initial volume of the gelatine solution.

Statistical analysis

The data were reported as three independent experiments' mean ± standard deviation. The analysis of variance was used to determine the statistical significance of the quadratic polynomial model equation (ANOVA). An ANOVA study with a 95% confidence level was performed to analyse the effect of each output (temperature, time, and concentration) on dependent parameters to test the anticipated model on the response variables. The desirability function approach was used to find the ideal extraction conditions for achieving the maximum values of the four responses.

Results and discussion

Chemical composition of raw material

Chicken by-product mixture had moisture as the major component of 66.293 ± 0.282%. The ash content of the raw material mixture (5.176 ± 0.580%) was slightly higher than that reported by Du *et al*. (2013) for chicken heads (4.64%). Protein content (21.55 ± 0.599%) was much lower than that found by Araújo *et al*. (2018) in the chicken feet (77.59%), probably because the protein content in that study was reported based on dry matter. However, the protein content in Widyasari and Rawdkuen (2014) study was 18.69%, lower than that reported in the present work. Fat content reached a high value of 6.686 ± 0.224%. Blending both heads and feet by-products seemed to increase some chemical components, thus influencing gelatine's extraction yield and composition.

Model fitting

Table 1 summarises the experimental design and the mean values of different responses, each with three levels of gelatine yield, viscosity, emulsifying activity, and foaming capacity. The extraction was optimised by applying second-order polynomial equations based on predefined process parameters. All the coefficients of the linear (X1, X2, X3), quadratic ($X1^2$, $X2^2$, $X3^2$), and interactions (X1X2, X1X3, X2X3) were calculated for their significance. Table 2 lists the coefficients of the regression models of Y1, Y2, Y3, and Y4 and their specified determination coefficients (R^2) for yield, viscosity, emulsifying activity, and foaming capacity which were 90.13, 98.63, 98.78, and 91.62%, respectively. According to Guan and Yao (2008), a good adjustment requires an R^2 of 80% which was compatible with our determination coefficient R^2.

Table 1. Taguchi experimental design and mean values of different responses.

N	X1	X2	X3	Y1	Y2	Y3	Y4
1	55	2	2	2.23	4.88	30.41	26
2	55	2	3.5	2.99	3.89	26.16	41
3	55	2	5	5.33	3.06	23.54	46
4	55	4	2	4.35	4.65	29.68	34.25
5	55	4	3.5	4.71	3.88	25.89	45
6	55	4	5	4.97	3.06	24.08	48.75
7	55	6	2	5.74	4.38	29.17	38.75
8	55	6	3.5	6.7	3.74	25.74	46.25
9	55	6	5	7.45	3.01	22.6	66.5
10	65	2	2	3.35	4.24	29.15	26
11	65	2	3.5	4.76	3.7	25.31	42.5
12	65	2	5	6.29	2.86	22.52	47.25
13	65	4	2	3.6	4.24	28.77	26
14	65	4	3.5	6.09	3.62	25.24	39.25
15	65	4	5	6.77	2.53	20.52	57.5
16	65	6	2	6.05	4.04	27.48	30
17	65	6	3.5	6.9	3.53	25.59	42.5
18	65	6	5	7.34	2.46	20.46	59.5
19	75	2	2	5.15	4.02	27.27	21.25
20	75	2	3.5	5.38	3.4	24.06	39.75
21	75	2	5	6.12	2.39	19.17	73.75
22	75	4	2	7.34	3.98	27.54	26
23	75	4	3.5	8.99	3.19	23.77	39.75
24	75	4	5	10.27	2.09	19.48	87.75
25	75	6	2	8.16	3.93	26.1	33.75
26	75	6	3.5	12.92	3.18	23.6	43.75
27	75	6	5	13.84	2.02	19.28	95

X1 = extraction temperature (°C), X2 = extraction time (h), X3 = acetic acid concentration (%), Y1 = yield (%), Y2 = viscosity (mPa.s), Y3 = emulsifying capacity (m^2/g), and Y4 = foaming capacity (%).

Table 2. Regression coefficient (a), coefficient of determination (R^2), and *F*-test values of the regression model.

Regression coefficient (a)				
	Yield	**Viscosity**	**Emulsifying capacity**	**Foaming capacity**
Intercept				
X0	49.5	7.25	39.73	405
Linear term				
X1	-1.511***	-0.045***	-0.069***	-9.92*
X2	-1.93***	-0.090***	-0.646***	-0.86***
X3	-0.07***	0105***	-1.267***	-46.4***
Quadric term				
$X1^2$	+0.011*	0.001	-0.00029	0.0062*
$X2^2$	+0.035	0.0035	0.016	0.153
$X 3^2$	-0.0112	-0.06***	-0.0069	2.09
Interaction term				
X1*X2	+0.037*	0.00008	0.0054	-0.031
X1*X3	+0.023	0.0036	-0,015	-0.629***
X2*X3	+0.046	0.0008	-0.019	0.687
R^2	90.13	98.63	98.78	91.62
F-value (model)	169.814	14.9445	266.224	7910.71
Error	18.588	0.2068	6.408	723.52

Level of significance: * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$. X1 = extraction temperature (°C), X2 = extraction time (h), and X3 = acetic acid concentration (%).

A negative linear effect of temperature (X1) was significant on both yield, viscosity, and emulsifying and foaming capacity, as well as time (X2) and acetic acid concentration (X3). The quadratic effect of X1 produced a significant positive effect only on yield and foaming capacity, whereas $X2^2$ exerted a highly negative effect on viscosity. The interaction effect of X1*X2 (temperature*time) was found to be significant only on yield, and X1*X3 (temperature*acetic acid concentration) was very highly significant only for foaming capacity (Table 2).

Figure 1 depicts the ANOVA result for the percentage contribution of all process variables to each investigated response; for the yield response, the percentage contribution of temperature and time is more dominant, whereas, for the other responses, the percentage contribution of the acetic acid concentration variable is more significant.

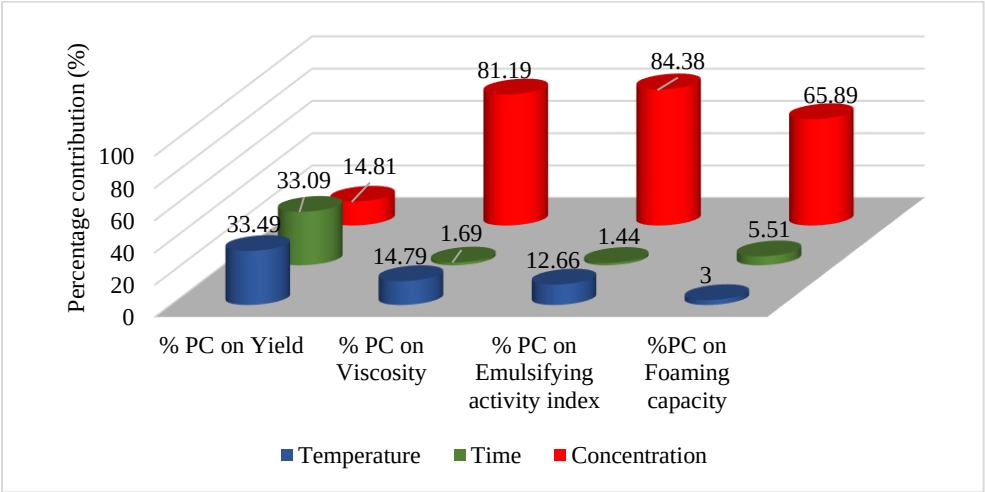

Figure 1. Percentage contribution (PC) of selected parameters on investigated responses. Y1 = yield (%), Y2 = viscosity (mPa.s), Y3 = emulsifying capacity (m^2/g), and Y4 = foaming capacity (%).

Effect of extraction variables on response parameters

A helpful graphical tool called the contours 2D response surface plot was employed to comprehend the interrelationship between the analysed responses and the selected parameters. This interdependence is illustrated by varying one dependent parameter against two independent ones while holding the other constant. Figure 2 depicts the 2D contours of the reaction, *i.e*., Y1, Y2, Y3, and Y4, as a function of X1, X2, and X3. The greatest gelatine yield retrieved from the feet-heads blend (13.84%) in the present work was higher than that obtained from chicken feet (Choe and Kim, 2018). The high yield (21%) obtained from the chicken heads study conducted by Du *et al*. (2013) was due to the calculation based on the dry weight of collagen in the raw material. Gelatine extraction yield variation between the different studies could be mainly due to the difference in extraction methods (Widyasari and Rawdkuen, 2015), which used different extraction times, pretreatment, and washing steps. The collagen content also affects the gelatine extraction yield, which depends on the raw material.

Based on Figure 2a, extraction yield was positively influenced by higher temperature and time extraction. This observation was the same as that of Jakhar *et al*. (2014) and Kim (2017). The higher temperatures used for gelatine extraction destroyed the hydrogen bonds stabilising the collagen structure, thus contributing to an efficient denaturation of this later, and a higher gelatine yield (Omar and Sarbon, 2016). A prolonged extraction period would increase collagen denaturation, and favoured gelatine extraction. The gelatine viscosity decreased when the value of the predefined parameters increased. As shown in Figure 2b, the acid concentration and extraction time impact on viscosity was significant ($p < 0.05$). Gelatine solution viscosity was negatively affected by acetic acid concentration and extraction temperature. Sompie and Triasih (2018) reported that acetic acid breaks peptide bonds of amino acids into short-chain molecules which reduces viscosity, but high extraction temperatures, as reported by Pradarameswari *et al*. (2018), led the amino acid structure to an opening chain, thus resulting in a shorter one, and reduced gelatine viscosity. In addition, viscosity is partially influenced by molecular weight, which is affected by the hydrolysis of peptide chains, and thereby, gelatine recovery (Sarbon *et al*., 2013). Overall, viscosity values in the present work ranged between 2.02 and 4.88 mPa.s, which was consistent with the range reported for commercial gelatine viscosity (2.0 to 7.0 mPa.s) (Johnston-Banks, 1990). The gelatine recovered from chicken heads-feet blend by-product seemed to belong to the high-quality food and pharmaceutical gelatines category, whose viscosity ranges between 2.0 - 7.5 mPa.s (Mokrejš *et al*., 2019). Finally, the optimal value of viscosity (3.36 mPa.s) was higher than that found for chicken feet gelatine (Widyasari and Rawdkuen, 2014), but lower than gelatine from chicken heads (Ahmed, 2017; Gál *et al*., 2020).

Different samples' emulsifying activity index (EAI) ranged from 19.17 to 30.41 m^2/g, higher than that found for gelatine from chicken feet (Chakka *et al*., 2017). Hydrophobic areas on the peptide chains of gelatine allow it to emulsify and foam (Damodaran, 2006). The concentration of acid used in gelatine extraction, and characterisation of the raw material had an impact on the emulsifying capacity of the extracted gelatines (Khiari *et al*., 2013; Chakka *et al*., 2017). Figure 2c shows lower acetic acid concentrations and extraction temperatures produced higher emulsifying activity index. These results were in good agreement with those reported by Chakka *et al*. (2017) who demonstrated that the EAI of chicken feet gelatine decreased with increasing acid concentration during extraction. Highly oxidised proteins were thought to have reduced emulsifying activity (Chakka *et al*., 2017).

The foaming capacity (FC) is mainly determined by the properties of the raw components (Gómez-Guillén *et al*., 2011). To produce foam at an air-water interface, the molecules must have hydrophobic zones interacting with the water surface (Townsend and Nakai, 1983). The differences in the ability to foam are caused by differences in the amount of hydrophobic amino acids, namely: alanine, valine, isoleucine, leucine, proline, methionine, phenylalanine, and tyrosine (Sarbon *et al*., 2015; Zilhadia *et al*., 2018). The different factors influencing foam formation at the air-water interface are transport, penetration, and rearrangement of the protein molecule. In order to display good foaming ability, a protein must be able to migrate quickly to the air-water contact, unfold, and reorganise at the interface (Halling, 1981).

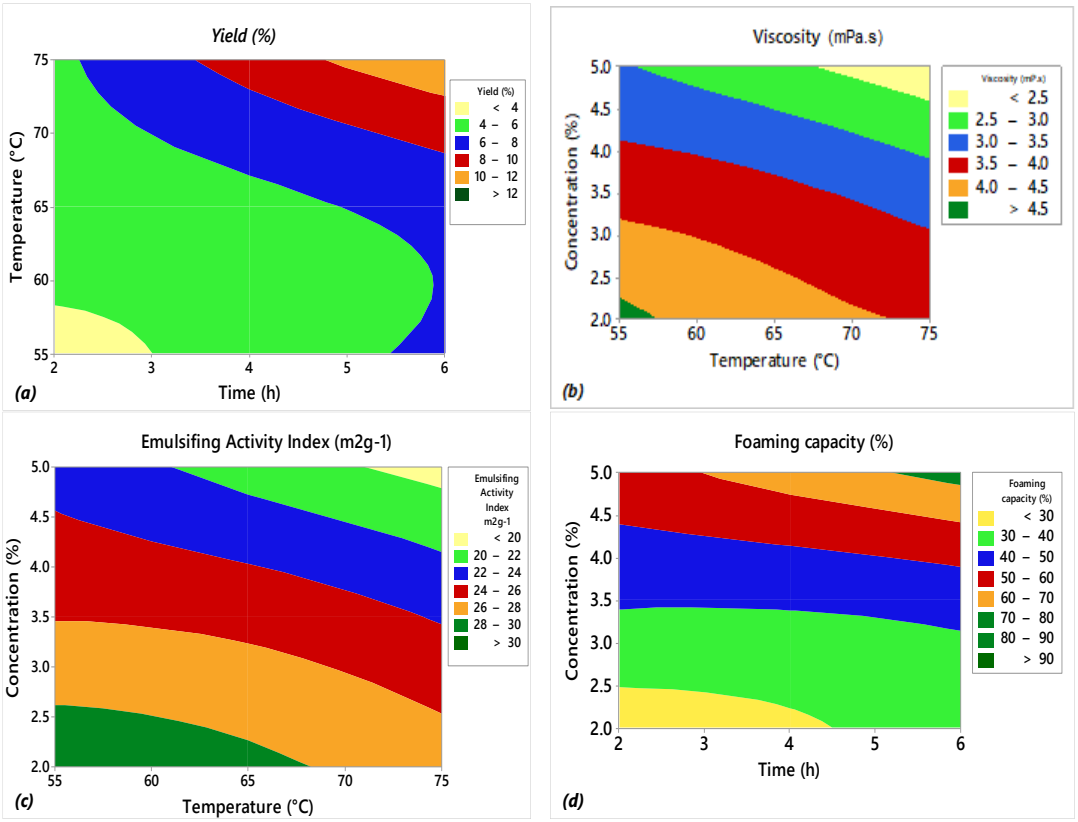

Figure 2. Response 2D contour plot of **(a)** yield, **(b)** viscosity, **(c)** emulsifying activity index, and **(d)** foaming capacity.

Figure 2d shows that acetic acid concentrations significantly influenced the foaming capacity. The foaming capacity increased with increasing acetic acid concentration, as similarly observed by Chakka *et al*. (2017). The increase in acetic acid concentration could probably generate more hydrophobic amino acids, thus contributing to an increase in foaming capacity in the present work. Time extraction seemed also to impact the foaming capacity positively. According to Ismail *et al*. (2019), the longer the extraction time, the better the foaming capacity of gelatine extracted from silver catfish, which was similarly observed in the present work, where the maximum FC (95%) was obtained with the higher time extraction (6 h) and higher acetic acid concentration.

Validation of RSM-based regression models

To assess the constructed model's validity, it is necessary to check its soundness fit. Hence, residual plots for models Y1, Y2, Y3, and Y4 (Figure 3a - 3d) and comparing the actual and predicted values were performed. It was possible to see a close match between the actual and predicted values. Overall, it can be highlighted that the derived models in the present work were perfectly fitted.

RSM-based models optimisation using the desirability function (DF)

In the present work, the desirability function (DF) was used for performing the optimisation task. The desirability function has been classified as one of the well-known adopted techniques in the industry that helps to determine the ideal solution in a multi-criteria process (Hertz and Kobler, 2000). In the DF approach, the processing parameters set with the highest desirability value are primarily chosen as the ideal solution (Mia and Dhar, 2016). Design-Expert (10) software was used to perform the DF in the present work. The process parameters (X1, X2, and X3) were kept within the experimental range, while the highest values of Y1, Y2, Y3, and Y4 were selected.

The desired goals, the interval of examined process parameters, and the realised perfect solution were given at a greater desirability value (0.476). DF provided the appropriate technical parameters of 75°C temperature, 6 h extraction duration, and 3.06%

acetic acid concentration. After processing this combination, the optimal values of the analysed responses were 10.97%, 3.36 mPa.s, 24.22 m2/g, and 45.07% for Y1, Y2, Y3, and Y4, respectively. In addition, a confirmation test was prepared and carried out to ensure the applicability of the best solution obtained during the optimisation phase based on the proposed ideal independent parameter configuration supplied by DF 0.467. Table 3 lists the percentage differences between predicted and experimental measured values for Y1, Y2, Y3, and Y4. The range variations were within 5%, which could be acceptable.

Figure 3. Comparison of actual and predicted values of **(a)** yield, **(b)** viscosity, **(c)** emulsifying activity index, and **(d)** foaming capacity.

Table 3. Comparison of optimal solution suggested by DF with confirmation test.

	Independent parameter			Dependent parameter			
	X1 (°C)	X2 (h)	X3 (%)	Y1 (%)	Y2 (mPa.s)	Y3 (m^2/g)	Y4 (%)
DF	75	6	3.06	10.97	3.36	24.22	45.07
Experimental test	75	6	3	10.75	3.18	23.22	46
Percentage deviation from experimental test				0.37	5.36	4.13	2.11

DF = desirability function, X1 = extraction temperature (°C), X2 = extraction time (h), X3 = acetic acid concentration (%), Y1 = yield (%), Y2 = viscosity (mPa.s), Y3 = emulsifying capacity (m^2/g), and Y4 = foaming capacity (%).

Conclusion

To our knowledge, this is the first study to focus on using the chicken by-product mix to extract the gelatine. Combining chicken feet and head by-products can reduce waste sorting, and transform it into valuable gelatine. The goal of optimising gelatine extraction conditions from a mixed by-product was to maximise gelatine yield extraction with intriguing functional qualities for the food industry. The most efficient conditions for extracting gelatine from the chicken heads-feet combination were 3.06% acetic acid, 75°C, and 6 h of extraction from collagen. The findings could benefit the food industry, particularly

poultry slaughterhouses, which could valorise these by-products blended into gelatine using acetic acid, which in turn is less detrimental to the environment. Further research must examine this gelatine's physicochemical, rheological, and spectral features extracted from the heads-feet mix by-product, and its applications.

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