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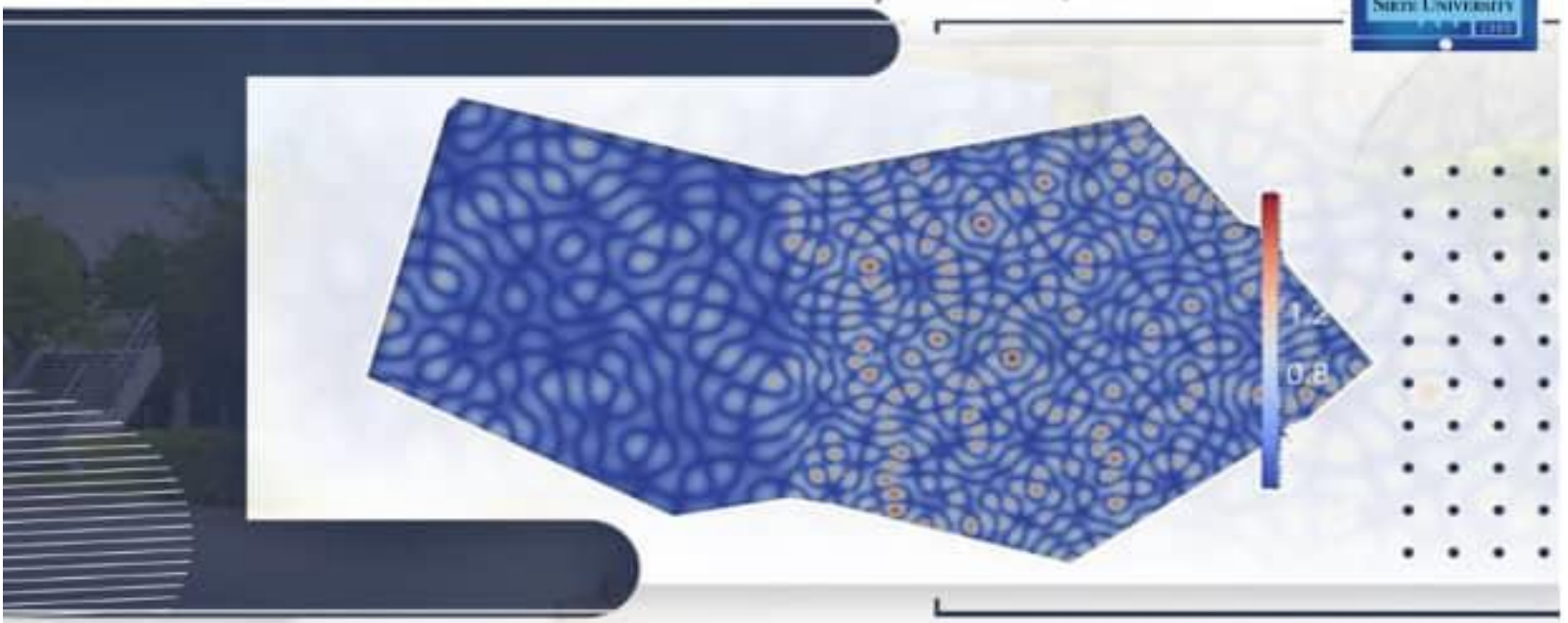

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Statistical Study of Extract Keratin Protein from Waste Chicken Feather Based on Response Surface Methodology

Dalal M. Ibrahim[1], Hanan F. Emrayed[2], Sumayyah M. Albarani[1], and Bushra Alnaji Abdalrazig[1]

[1]*Chemistry Department, Science Faculty, Omar Al-Mukhtar University, Libya.*

[2]*Chemical Engineering Department, Engineering Faculty, Omar Al-Mukhtar University, Libya.*

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A B S T R A C T

The feathers contain a significant amount of keratin protein, which is used in cosmetics, shampoos, hair treatment creams, and skin creams. Dissolving chicken feathers with reducing agent and then separating the protein from chemicals are the key steps involved. However, in order to enhance the amount of recovered keratin as much as possible, the best conditions for extracting keratin from chicken feathers are required. In this study, Response Surface Methodology (RSM) was used in order to simulate and optimize the operating parameters for extracting keratin from waste chicken feathers in order to increase the amount of keratin protein compared to previous studies. Dissolving chicken feathers using sodium sulphide as a reducing agent at various periods, temperatures, and concentrations is the first step in the fundamental technique. After the feathers have been dissolved with a reducing agent, the fluid is treated with an ammonium sulfate solution to precipitate the protein. As determined by a biuret test and UV-Vis analysis, the keratin protein had a maximum wavelength of 290 nm. Finally, the statistical optimization of the extraction conditions provided a better understanding of the reaction parameters. The optimum yield of keratin was achieved at 3.7 hours at 30.07°C with 0.05 M sodium sulfide.

1 Introduction

Many countries had a large poultry slaughterhouse sector that discarded about four million tones f chicken feathers each year, and they took advantage of the circumstance to conduct research and find ways to monetize the waste. There is a growing interest in the creation of environmentally friendly, renewable-resource-based materials. The current research is the first in Libya to recycle chicken feather waste, and it focuses on extracting natural keratin protein from chicken feathers with the help of a reducing agent. Reducing substances aid in lowering the stability of keratin fibers in their solid state, which is present in feathers. In order to dissolve keratin fibers into protein solutions, these chemicals will break down disulfide bonds, hydrogen bonds, and salt linkages. Feathers make up over 90% of the keratin protein in waste biomass, as previously stated (Fakhfakh-Zouari, Haddar, Hmidet, Frikha, & Nasri, 2010; Gessesse, Hatti-Kaul, Gashe, & Mattiasson, 2003; Grazziotin, Pimentel, De Jong, & Brandelli, 2006; Saucedo-Rivalcoba, Martínez-Hernández, Martínez-Barrera, Velasco-Santos, & Castaño, 2011).

A-keratins and b-keratins are the two most common types of keratins (Barone, Schmidt, & Gregoire, 2006; Sharma & Gupta, 2016). In mammals, a-keratins are found in abundance, while b-keratins are found in abundance in birds and reptiles. A-keratins may be found in mammals' hair, wool, horns, nails, claws, and hooves, while b-keratins can be found in reptiles' nails, scales, claws, shells, feathers, beaks, and quills (Ng et al., 2012). The reductants function quickly and without creating any chemical changes or a reduction in protein yield. The

Corresponding author: E-mail: dalal.m.ibrahim@omu.edu.ly

solutions' by-products act like true proteins, not hydrolysis by-products. Sulfosalicylic acid and ammonium sulfate, two common protein precipitants, are employed to precipitate their solutions. Keratin is well-known for its ability to stretch in a variety of directions without breaking and for forming a strong, fibrous matrix in tissues (Yamauchi & Yamauchi, 2002).

According to Leichner. (2019), chicken feather keratins can be converted to natural protein that is soluble in alkali or acid and digestible by trypsin and pepsin. To achieve this, the disulfide bonds in keratin were broken. Keratins in feathers are made up of twisted, pleated sheets that are then stabilized and toughened by disulfide bonds.The strength of the keratin in chicken feathers can be reduced by disrupting these disulfide connections, allowing the keratin to become soluble and convert to natural protein (Leichner et al., 2019).

In the case of Schrooyen et al, oxidizing chemicals like bromine, permanganate, and hydrogen oxide break disulfide bonds very slowly, slowing down the protein extraction process. On the other hand, the reducing agents operate swiftly and dissolve keratin only in alkaline reactions (pH 10 to 13), but their activity is not solely due to alkali. The results of these processes behave like real proteins, not hydrolysis byproducts. Schrooyen et al explored the effect of adding varying amounts of SDS on the rate of aggregation of polypeptide chains and the rate of oxidation of cysteine residues during dialysis, as have a few other researchers (Schrooyen, Dijkstra, Oberthür, Bantjes, & Feijen, 2001). Yamauchi et al. studied urea, 2-mercaptoethanol, (Kamarudin et al., 2017)solution (sodium dodecyl sulphate). They discovered that using SDS as a surfactant sped up the extraction process and boosted the yield. It also stabilized the aqueous protein solution following urea removal via dialysis against 2-mercaptoethanol-containing water (0.08 wt percent). The surfactant binds to keratin and is eliminated by dialysis significantly more slowly than other low-molecular-mass substances (Yamauchi & Yamauchi, 2002).

Many countries had a large poultry slaughterhouse sector that disposed of approximately four million tonnes of chicken feathers per year, and they took advantage of the circumstance to conduct research and find ways to monetize the waste. Currently, there is a growing interest in the production of environmentally friendly materials derived from renewable resources.The current study is the first in Libya to recycle chicken feather waste, and it focuses on extracting natural keratin protein from chicken feathers using a reducing agent and methodically optimizing extraction conditions. The present research was undertaken to extract natural keratin protein from chicken feathers by using different reducing agents. The reducing agent helps in decreasing the stability of keratin fibers in the solid form found in feathers. These reagents will break down disulfide bonds, hydrogen bonds, and salt linkages of the keratin fibers in order to dissolve them into protein solution. In this study, keratin is extracted on a lab scale using sodium sulfide as a reducing agent. RSM is used to optimize the keratin extraction process to determine the best parameters to increase the amount of keratin.

2 Response Surface Methodology

RSM is a set of statistical and mathematical approaches used in the chemical field to investigate the impacts of a variety of variables and how their interactions affect the desired response (Kamarudin et al., 2017). Used central composite design (CCD) to build the input parameters in this study because it produces more precise prediction findings than other methods. Figure (1) shows the estimating procedure for this method

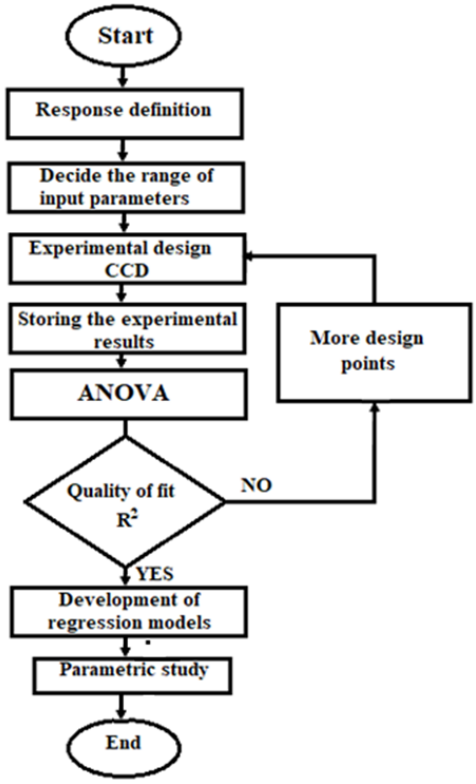

Figure (1). Response Surface Methodology (RSM) Flow Chart.

An ANOVA is used to examine the relationship between the factors and the responses. The significant factor is represented by the "P-value" statistic in the analysis of variance (ANOVA) table of the model and its terms, which should be less than 0.05. The smaller the 'P-value," the more significant the result (Welu, Beyan, Balakrishnan, & Admassu, 2020).

3 Materials and Methods.

3.1 Chemical Tools and Materials of the Experiment

The utilized glassware is conical flask, beakers 250ml - Filter paper, burette, graduated cylinder, weightier, and heater, while the materials that are used in the extraction are shown in Table (1).

Table (1). The Experiment Materials

Chemicals	Molecular Formula
Sodium sulphide	Na2S
Ammonium sulphate	(NH4)2SO3
Sodium hydroxide	NaOH
Copper sulphate	CuSO4
Potassium hydroxide	KOH

3.2 Feathers before treatment

Feathers from poultry processing are collected and steeped in ether for 24 hours. The major goal is to remove stains, oil, and grease from the feathers before processing them. After that, the feathers are cleaned in soapy water and dried in the sun. After that, the dried feathers are mixed and properly stored in a sealed plastic bag.

3.3 Chicken Feathers Dissolving

According to RSM, the sodium sulfide solutions are changed in a range of 0.5-1M, the temperature range is 30° to 80°C, and the heating and spinning time is changed in a range of 2-6 hours. Sodium sulfide solutions are prepared in molarity in a 1 liter conical flask. Weigh and add 10 g of mixed chicken feathers to the sodium sulfide solution. The solution is heated while the PH is kept at about 10–13, and the solution is swirled continuously. The solution is then filtered and centrifuged for 5 minutes at 10,000 rpm. To remove particles, the supernatant liquid was carefully collected and filtered using filter paper. In 0.5 L of deionized water, 20 g of ammonium sulfate is dissolved. Stir the solution until all of the ammonium sulfate particles have been dissolved. The solution is subsequently filtered to remove any remaining particles.

3.4 Precipitation of proteins

The feather filtrate solution had been collected and mixed in a beaker previously. Ammonium sulfate solution is added drop by drop. Feather filtrate and ammonium sulfate solution are used in a 1:1 ratio. The solid particles are carefully recovered after centrifuging the solution for 5 minutes at 20,000 rpm. The supernatant liquids are collected.

3.5 Protein Purification

100 mL deionized water is added to the recovered solid particles (washing). The solution is centrifuged at 20,000 rpm for 5 minutes, and the solids are carefully collected. The solid particles are then dissolved in a 100 mL sodium hydroxide solution at a concentration of 2 M. All of the liquids are carefully collected and kept after centrifuging the solution at 20,000 rpm for 5 minutes, while the solids are discarded. The precipitating, washing, and dissolving steps are repeated three times each.

3.6 Biuret Test

A copper sulphate solution of 1% and a potassium hydroxide solution of 1% are made. In a 1:1 ratio, 5 mL of the collected solution is combined with potassium hydroxide solution. To the mixed solution, three drops of copper sulphate solution are added. Observed and documented changes in the solution. The absorbance of the solution is measured using UV-Vis.

3.7 RSM Procedure Process

The Design-Expert software version 7.0 was applied to design the experiments based on the Central Composite Design (CCD) and then study the influence of the experimental parameters on the amount of produced protein. (Table 2) shows the selected input parameters and their levels that were used to identify the parameters of the production of the keratin protein. In Table (2) (-1), (0), and (+1) were chosen to indicate the lowest, central, and highest level, respectively. Three experimental parameters were investigated: design parameter A was the Na2S, design parameter B was temperature, and design parameter C was time. Moreover, the responses in this study were the amount of keratin protein.

Table (2). Independent parameters considered in this study and their levels for central composite design

Parameter	-1	0	+1
Na2S(M)	0.5	0.75	1
Temperature (Co)	30	55	80
Time (hr)	2	4	6

Optimization was applied using the desirability profile and its functions in the RSM. The input parameters with high desirability were chosen as the final experimental parameters for producing keratin protein. The target was to maximize the amount of keratin protein and minimize the experimental time, with the Na2S, and temperature being set in a certain range for satisfactory results within the upper and lower limits. The solution with high desirability was preferred. Table (3) shows the target value and the upper value for all the parameters.

Table (3). Target value and limit for optimization of the Experimental parameter

Experimental parameter	Target	Lower limit	Upper limit
Na2S(M)	In a range	0.5	1
Temperature(Co)	In a range	30	80
Time	Minimize	2	6

4 Results and Discussion

4.1 Observation

In a sodium sulfide solution, chicken feathers totally dissolve. The biuret test confirms the presence of protein. After the reagent is added, the solution turns purple, which is only feasible if peptide bonds are present. The purple color increases as the amount of peptide bonds increases. Keratin had previously been removed, according to the results of a biuret test and a UV-visible spectrophotometer. The biuret test, as shown in Figure (2), showed the existence of protein, and the display of the absorption spectra on the keratin solution revealed a value of λmax at 290 nm, as shown in Figure (3).

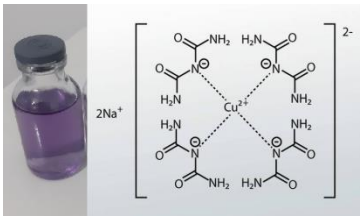

Figure (2). Biuret Test

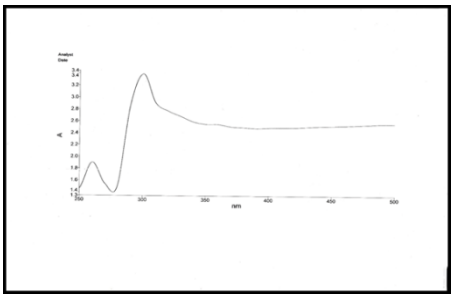

Figure (3). UV visible spectrum of extracted keratin

Absorbance is proportional to a solution's concentration. As the absorbance rises, the protein concentration rises as well. Because the solution is extremely alkaline, with a pH ranging from 10 to 13, the absorbance of feathers in sodium sulfide reactive solution is maximum, and the dissolving rate of feathers in sodium sulfide solution is high. The ionic bond formed by electrostatic attraction between the NHs+ group of di amino acids and the COO group of dicarboxylic acids can be disrupted in the alkaline state because the proton is removed from the amino group. These ionic bonds must be disrupted first for some reason in order to diminish the disulfide bonds of the keratin and disintegrate the feathers. The sodium sulfide solution is readily alkaline, but it requires the addition of sodium hydroxide to make it alkaline with a pH of 10 to 13. Protons cannot be removed without an alkaline state, resulting in the ionic link being broken. As a result, sodium hydroxide plays a critical role in the dissolution of feathers.

4.2 Statistical Investigation of Extraction Parameters of Keratin Using RSM

The Na2S, temperature, and time were changed based on the central composite design (CCD). The responses in this study were to the amount of keratin protein. The arrangement of the central composite design, responses, and their values from the experimental results of different parameters. A total number of fifteen experimental were executed and the responses are listed in Table (4).

Table (4). Central composite design arrangement, responses and their values for experimental results of amount of Keratin protein.

Run	Independent factors			Response
	A: Na2S (M)	B: Temperature (C°)	C: Time (hr)	Amount of Keratin Protein
1	1	30	6	3.3
2	0.75	55	4	3.4
3	0.75	55	2	2.9
4	0.50	55	4	3.8
5	0.75	55	6	3.4
6	0.75	55	4	3.4
7	0.75	55	4	3.4
8	1	80	2	2.7
9	0.50	30	2	4.9
10	0.50	80	6	2.4
11	0.75	55	4	3.4
12	1	55	4	3.0
13	0.75	55	4	3.4
14	0.75	30	4	3.9
15	0.75	80	4	2.9

Experimental results reflected that the amount of keratin protein varied between (2.4-4.9) as seen in Table (4). The analysis of variance (ANOVA) can be seen in Table)5) which shows the independent variables, the Na2S, and the time that were significant ($p < 0.05$). Additionally, the interaction impact of the Na2S, and the temperature were significant as the p-value was equal to < 0.0001, while, the temperature, the interaction impact of the Na2S, and the time, and the interaction impact of the temperature, and the time were insignificant as the p-values were equal to 0.1499, 0.024 and 0.47, respectively.

Table (5). ANOVA table for Amount of Keratin Protein response in surface quadratic model.

Source	Mean Square	F value	P-value Prob > F	Remarks
Model	0.78	59.43	<0.0001	Significant
A-Na2S	0.20	15.31	0.0045	Significant
B-Temperature	0.033	2.54	<0.1499	Not Significant
C-Time	0.22	16.67	0.0035	Significant
AB	0.70	53.48	<0.0001	Significant
AC	0.10	7.69	0.024	Not Significant
BC	0.0075	0.57	0.47	Not Significant

The impact of the independent parameters on the responses was illustrated graphically in terms of 3-D surface plots and can be seen clearly below. These graphs were drawn by fixing one independent parameter while the other two parameters being used to determine the response were left untouched. Figure (4) shows the amount of keratin protein against AB. The graph shows that any decrement in the Na2S would have some negative effects on the response, while temperature would have clear positive effects on the response. However, the decrement of both parameters would have clear negative effects on the response. That means the effect of the interaction of both parameters is very high.

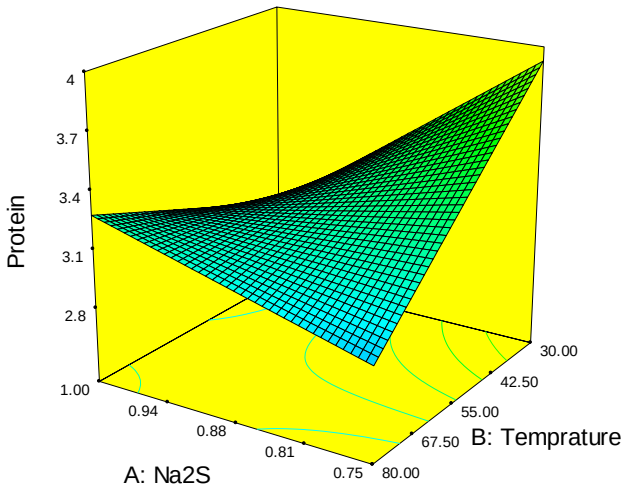

Figure)4). 3 D surface plote for Influence of Na2S and temperature in the amount of keratin protein

Figure (5) shows the amount of keratin protein against AC, and the graph shows that any decrement in the Na2S would have some positive effects on the response, while the increment in the time showed significant positive effects on the response. The decrement of Na2S and the increment of time would have clear positive effects on the response. That means the effect of the interaction of both parameters is low.

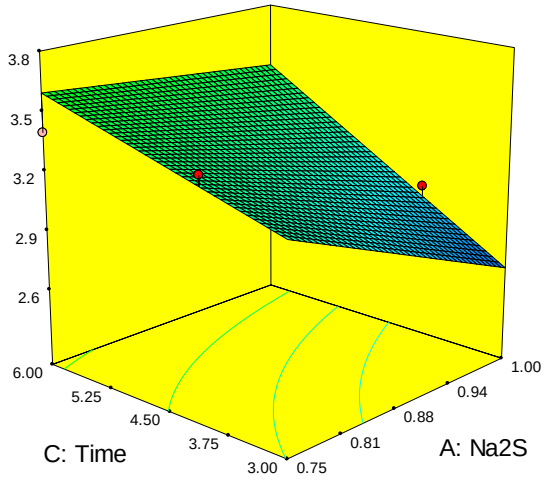

Figure (5). 3 D surface plote for Influence of Na2S and time in the amount of keratin protein

Figure)6) shows the amount of keratin protein against BC, and the graph shows that any decrement in the temperature would have some positive effects on the response, while the increment in the time showed significant positive effects on the response. The decrement of temperature and the increment of time would have clear positive effects on the response. That means the effect of the interaction of both parameters is low.

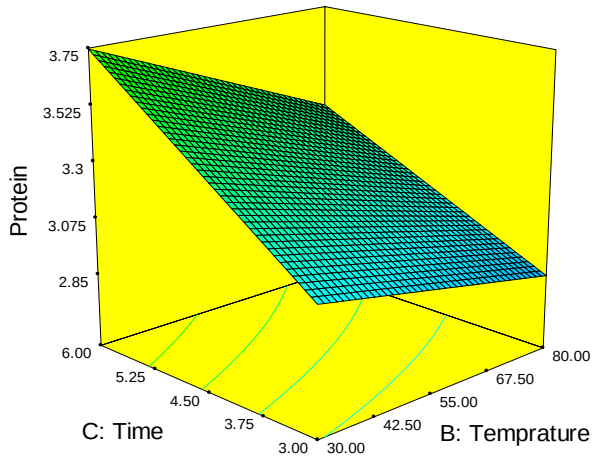

Figure (6). 3 D surface plot for Influence of time and temperature in the amount of keratin protein.

4.3 Optimization of the Experimental parameter

RSM was used in this work to optimize the independent parameters of producing keratin protein. The primary benefit of utilizing the response surface methodology (RSM) is that the amount of protein can be enhanced by regulating the input parameters. The optimization analysis was established by the desirability analysis in Equation (1) (Welu et al., 2020). It was not necessary that the desirability value should be 1.0 as the value was completely dependent on how closely the lower and upper limits were set relative to the actual optimum values.

$$D = (d_1 x d_2 x \dots\dots x d_n)^{1/n} = (\Pi_{i=1}^{n} d_i)^{1/n} \qquad (1)$$

Where D was the overall desirability ranging from 0 to 1 and n was the number of responses. The extracted keratin protein with parameters that had the highest desirability was chosen as a ideal experimental that has high amount of protein. The optimum condition for all case studies were achieved when the amount of protein was maximized with the highest desirability value of 1. From Figure 7, it can be clearly seen that RSM calculated the amount of protein was at maximum when the parameters of the experimental were as follows: NS2A was 0.5 M, the temperature was 30 C°, and the time was 3.7 hr.

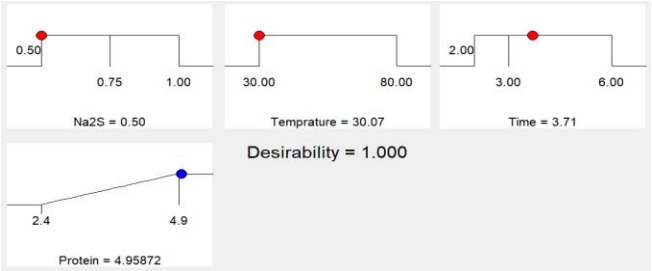

Figure (7). Ramp function graph of desirability for optimization solution for Amount of Keratin Protein

The bar graph and ramp function graph of Figure 7 show the desirability values for output responses. The dot on each ramp reflected the factor setting or response prediction for that particular response characteristic. The height of the dot shows how much desirable it was compared to the baseline. A linear ramp function was created between the low value and the goal or the high value and the goal, as the weight for each parameter was set equal to one.

5 Conclusions

The aim of this research was to locate a different source of keratin protein. Chicken feathers are a hazard for the environment since they contain crude protein and take a long time to decompose. The chicken feathers were first dissolved with reducing agents, and then protein was precipitated out of the solution with ammonium sulfate in this experiment. The presence of the protein was initially validated by the biuret test, in which the reagent turned purple in the presence of peptide bonds, while the absence of keratin protein was proven by the value of max. Finally, statistical adjustment of the extraction conditions resulted in a better understanding of the reaction parameters as well as a high keratin yield. The extraction process of keratin can be scaled up from the laboratory to the industrial level.

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Conflict of Interest: The authors declare that there are no conflicts of interests.

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