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Optimization of obtaining process of acid silage from poultry carcasses by response surface methodology

Otimização do processo de obtenção de silagem ácida de carcaças de frango por metodologia de superfície de resposta

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Highlights

RSM was used to evaluate the interrelationships between the variables of acid silages.
Acids influence the chemical composition, storage, and revolutions.
Silages should be stored for a minimum period of 20 days, stirred once a day.

Abstract

This study aims to optimize the process of obtaining acid silage from poultry carcasses by response surface methodology. For poultry silage preparation, dead animals from the production process in a broiler commercial farm were used. The carcasses were ground in an electric grinder, homogenized, and placed in 45 polyethylene containers with a capacity of 2 kg each, and distributed in a 3^3 incomplete factorial design of response surface methodology, with 15 treatments and three repetitions at the central point. The independent variables were acid concentration (X_1), days of storage (X_2), and daily number of turnings (X_3). The levels of the independent variables were X_1 =3, 5, and 7 (phosphoric and acetic acids in a ratio of 4:6); X_2 =10, 20, and 30 days of storage; and X_3 =0, 1, and 2 daily turnings. The dependent variables evaluated were pH, crude protein, lipid oxidation, oil extraction, and mesophile counting. The pH of the ensiled mass was influenced mainly by X_1 presenting a negative linear effect

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and positive quadratic effect. The storage time had a positive effect on pH. For crude protein, a negative linear and quadratic effect of X_1 were observed, indicating a region of maximum protein values at the midpoint. Regarding lipid oxidation, the model indicated a region of minimum values near the midpoint. The generated model for oil extraction indicated maximum values when the silage was stored for long periods and with high acid concentrations. For mesophilic count, various interactions among variables were observed by the generated model, and the response surface indicated a region with the highest microorganism number in low acid concentrations and after a few storage days. The response surface methodology allowed for the optimization of the variables (acid concentration, storage time, and daily turning number) in the preparation of poultry carcass silages. The best acid concentration that represented the optimal pH was 5% (2% phosphoric acid, 3% acetic acid), requiring storage for at least 20 days with only one daily turning.
Key words: Acetic acid. Phosphoric acid. Poultry waste.

Resumo

O objetivo desse trabalho foi a otimização do processo de obtenção de silagem ácida de carcaças de frango por metodologia de superfície de resposta. Para o preparo da silagem de frango, foram utilizados animais mortos durante o processo de criação em granja comercial. As carcaças foram moídas em moedor elétrico, homogeneizadas e colocadas em 45 recipientes de polietileno com capacidade de 2kg cada, distribuídas em delineamento fatorial incompleto 3^3 da metodologia de superfície de resposta, com 15 tratamentos e 3 repetições no ponto central. As variáveis avaliadas foram concentração de ácidos (x_1), tempo de armazenamento em dias (x_2) e número de revolvimentos diários da massa ensilada (x_3). Os níveis das variáveis foram: x_1 = 3, 5 e 7% (ácido fosfórico e ácido acético na proporção 4:6); x_2 = 10, 20 e 30 dias de armazenamento e x_3 = 0, 1 e 2 revolvimentos diários. as variáveis dependentes avaliadas foram o pH; proteína bruta; oxidação lipídica; extração de óleo e contagem de mesófilos. O pH da massa ensilada foi influenciado principalmente por x_1 apresentando efeito linear negativo e quadrático positivo. O tempo de armazenamento apresentou efeito linear positivo sobre o pH. Para proteína bruta foram observados efeitos lineares e, também, quadrático negativos de x_1, indicando uma região de máximos valores de proteína no ponto central. Na oxidação lipídica, o modelo indicou uma região de mínimos valores, próximo ao ponto central. O modelo gerado para extração de óleo indica máximos valores quando a silagem é armazenada por longos períodos e com altas concentrações de ácidos. Observou-se pelo modelo gerado para contagem de mesófilos, que a superfície de resposta indicou uma região com maior número de microrganismos em concentrações baixas de ácidos e poucos dias de armazenamento. A metodologia de superfície de resposta permitiu a otimização das variáveis: concentração de ácidos, tempo de armazenamento e número de revolvimentos diários, no preparo de silagem de carcaças de frangos. A concentração de ácidos que melhor representa os valores ideais de pH foi de 5% (2% ácido fosfórico, 3% ácido acético), sendo necessário o armazenamento por pelo menos 20 dias e com apenas um revolvimento diário.
Palavras-chave: Ácido acético. Ácido fosfórico. Resíduos de frango.

Introduction

The production and processing of poultry are characterized by a high volume of waste, such as litter, feathers, eggshells, carcasses, blood, and wastewater (McGauran et al., 2021). When improperly disposed of, the waste from poultry production and processing can pollute groundwater and surface waters, allowing the development of pathogenic microorganisms that may cause significant harm to human and animal populations as well as to the environment (Hubbard et al., 2020).

The waste and by-products generated during poultry processing contain natural polymers and valuable biological components (Galali et al., 2020; Kannah et al., 2020; Voběrková et al., 2020), which allow the application of methods that utilize them as feed, fertilizers, and new food ingredients (Belc et al., 2019). The methods applied to these wastes or by-products include traditional technologies, such as composting and incineration, or chemical (acid and alkaline treatments), physical (high-temperature, high-pressure, and ultrasonic treatment), and microbiological methods, resulting in biologically active compounds, lipids, and bioactive compounds (Borrajo et al., 2019; Chakka et al., 2015).

Methods like acid silage help reduce pathogenic microorganisms, making a product suitable for use in animal feed (Blake, 2004). The production of silage is a simple process consisting of three basic steps: grinding, acidification, and storage. Anaerobic storage conditions prevent oxidation and rancidity, a product does not produce odors, and refrigeration is not required, allowing it to maintain quality for a longer time (Vidotti & Gonçalves, 2006). The principle of the technique is pH reduction, which can be achieved by adding organic and/or mineral acids. With the pH reduction, enzymes naturally present or intentionally added promote protein hydrolysis. This process keeps the pH below 4, preventing microbial activity (Borghesi et al., 2007).

Several acids, either in combination or alone, can be used in acid silage production, such as formic acid and citric acid (Gao et al., 1992); formic acid and sulfuric acid (Vidotti et al., 2003); acetic acid (Seibel & Souza-Soares, 2003); muriatic acid (Beerli et al., 2004); and phosphoric acid and acetic acid (Vidotti & Gonçalves, 2006). Certain factors related to organic acids, such as hazard level, environmental and health risks, accessibility, and cost, must be considered for their use (Hisano & Borghesi, 2011). Silage can be used between 7 and 30 days after preparation, has a composition similar to the raw material, has high digestibility and the complete presence of amino acids, and can, therefore, be used as feed. Longer storage becomes a critical nutritional point, as amino acids and lipids begin to undergo changes (Oetterer, 1994).

Several studies have been conducted on ensiling poultry production or processing waste. Eissa et al. (2021) evaluated the effect of silaging chicken offal with betaine supplementation, and Rachmawati and Samidjan (2019) analyzed the inclusion of different levels of feather silage (0, 12.5, 25, 35.5, and 50%) as a replacement for fish meal. Fagbenro and Fasakin (1996) used fresh poultry viscera to prepare acid silage from chicken, adding citric and propionic acid, along with a commercial antioxidant (enthoyquin, 25 mg/kg) for fermentation. Middleton et al. (2001b) ensiled duck carcasses with the addition of sucrose and sweet potato residue, and the silage was used in the formulation of feed for growing tilapia, with no observed differences in fish palatability or growth.

Response surface methodology (RSM) is the most popular technique for process optimization, owing to its comprehensive theory, efficiency, and simplicity (Camilios et al., 2005). RSM consists of a group of statistical and mathematical procedures that can be used to study the interrelationships between one or more responses (dependent variables) and numerous factors (independent variables), defining the effects of the independent variables either individually or in combination, generating a model that describes a process (Diniz & Martin, 1996).

This study aimed to optimize the process of obtaining silage from chicken carcasses, using RSM, allowing for the utilization of chicken carcasses that die in commercial poultry farms.

Materials and Methods

Raw material

To prepare the silage, carcasses of broiler chickens that died during the rearing process at a commercial farm located in Cambé, Paraná, Brazil, were used. The collection of dead animals was performed daily, immediately after death, by the farm worker, and the carcasses were stored in a freezer. Subsequently, the carcasses were brought to the laboratory at the Fish Farming Station of the State University of Londrina for grinding, preparation, and silage production.

Silage production

The whole carcasses, without evisceration and feather removal, were thawed and ground in an electric meat grinder with an 8-mm sieve. The ground carcass product was mixed to obtain a homogeneous mass. This homogeneous mass, totaling 90 kg, was then divided into 45 batches and packed in polyethylene silos, which constituted the experimental treatments. Each silo received 2 kg of the homogeneous mass, along with phosphoric and acetic acids according to the treatments described in Table 1. A butylated hydroxytoluene antioxidant was added to the ensiled mass at 0.1% in a dilution of 20-g 100-ml^{-1} of ethanol, and 0.1% of an antifungal agent (sorbic acid) in a dilution of 10-g 100-ml^{-1} of ethanol (Vidotti & Gonçalves, 2006). The silos were identified and left to rest. The product was stirred with a rod according to the respective treatment (Table 1) to ensure proper mixing of the acid with the mass. The storage time of the silos followed the respective treatments (Table 1), and they were kept at room temperature, with an average minimum and maximum of 20.26°C and 31.79°C, respectively.

Table 1
Experimental design using three independent variables: concentration of the acid mixture (X_1), days of silage storage (X_2), and number of revolutions of the ensiled mass per day (X_3)

Treatments	Uncode Variables			Code variables		
	Acid concentrations* (%)	Storage time (days)	Revolutioons of the mass (number of time day^{-1})	X_1	X_2	X_3
1	3	10	1	-1	-1	0
2	7	10	1	1	-1	0
3	3	30	1	-1	1	0
4	7	30	1	1	1	0
5	3	20	0	-1	0	-1
6	7	20	0	1	0	-1
7	3	20	2	-1	0	1
8	7	20	2	1	0	1
9	5	10	0	0	-1	-1
10	5	30	0	0	1	-1
11	5	10	2	0	-1	1
12	5	30	2	0	1	1
13	5	20	1	0	0	0
14	5	20	1	0	0	0
15	5	20	1	0	0	0

* Phosphoric acid and acetic acid (4:6) v/v.

Chemical and microbiological analyses

For chemical analyses, approximately 150 g of samples were collected from each experimental unit after 10, 20, and 30 days of storage. Each experimental unit was homogenized before sample collection, to ensure uniformity. In the Animal Nutrition and Soil laboratories of the Departments of Animal Science and Agronomy at UEL, dry matter (DM), mineral matter, crude protein (CP), and ether extract were determined in duplicate, following Association of Official Analytical Chemists [AOAC] (2016) methods. Macromineral elements (Ca and P) were analyzed according to Malavolta et al. (1997).

To determine pH, a digital potentiometer, brand Hanna®, was used, with readings taken twice daily once in the morning and once in the afternoon throughout the experimental period. Lipid oxidation in the silage was assessed using the thiobarbituric acid reactive substances (TBARS) method (Sinnhuber & Yu, 1977). The standard used was 1,1,3,3-tetraethoxypropane, whose acidic hydrolysis generates malonaldehyde in a 1:1 mol ratio, allowing the results to be expressed in terms of the "TBARS value" and presented in mg of malonaldehyde kg^{-1} of sample. For oil extraction from the silage samples of each experimental unit, centrifugation was performed at 3,000 rpm for 30 min. The supernatant lipid fraction was measured using a graduated cylinder and pipette.

At the Laboratory of Inspection of Animal Products at the Department of Preventive Veterinary Medicine/UEL, microbiological analyses for the enumeration of mesophilic aerobes and most probable number (MPN) of coliforms at 30°C and 45°C were conducted. These analyses were performed at the time of the grinding chicken carcasses, considered day 0 (zero), before acid addition, and after 3, 10, 20, and 30 days of ensiling. The methodologies used were those described in Normative Instruction No. 62 of the Ministry of Agriculture (Instrução Normativa No. 62, MAPA, 2003).

Mesophilic aerobic microorganism count

A 25-g aliquot from each experimental unit was added to 225 mL of a 0.1% peptone saline solution, which was homogenized in a stomacher, and then serially diluted at a decimal scale, using a 0.1% peptone saline solution, from 10^{-1} to 10^{-7}. Next, 1 mL of each dilution was distributed into sterile Petri dishes and identified in duplicate. To each Petri dish, about 15–20 mL of Plate Count Agar was added, and after solidification, the plates were incubated at 36°C for 48 h (Instrução Normativa No. 62 (MAPA, 2003).

For the microorganism count, two plates from the same dilution with 25–250 colonies were selected, and the average of the number of colonies counted was multiplied by the corresponding dilution factor, expressed as the number of colony-forming units (CFU) per g of silage. For statistical analysis, the values were transformed into log CFU g^{-1} (Instrução Normativa No. 62 (MAPA, 2003).

MPN of coliforms (at 30°C and 45°C)

Presumptive test

Using the previously prepared dilutions from 10^{-1} to 10^{-7}, 10 mL of the 10^{-1} dilution was distributed into a series of three tubes containing double-strength lauryl sulfate broth (corresponding to the 10° dilution). Next, 1 mL of the 10^{-1} dilution was inoculated into a series of three tubes containing single-strength lauryl sulfate broth. The other chosen dilutions were also inoculated into a series of three tubes containing single-strength lauryl sulfate broth up to the 10^{-6} decimal dilutions (Instrução Normativa No. 62 (MAPA, 2003).

The tubes were incubated at 36°C for 24–48 h. Readings were taken after 24 h and repeated after 48 h when negative tubes were present. Tubes showing medium turbidity and gas in a Durham tube were considered coliform-suspect (Instrução Normativa No. 62 (MAPA, 2003).

Confirmatory test - coliforms at 30°C

Positive tubes containing lauryl sulfate broth were transferred to tubes containing brilliant green bile broth with 2% lactose and incubated at 36°C for 24 to 48 h. The presence of coliforms at 30°C was confirmed by the presence of gas in Durham tubes. To calculate the MPN of coliforms (MPN g-1), the Table in Annex III (Basic Counting Procedures), included in Instrução Normativa No. 62 (MAPA, 2003), was used.

Confirmatory test - coliforms at 45°C

Positive tubes containing lauryl sulfate broth were transferred to tubes containing *Escherichia coli* broth and incubated in a water bath at 45°C for 24 to 48 h. The presence of coliforms was confirmed by the gas in the Durham tubes. To calculate the MPN of coliforms (MPN g^{-1}), the Table in Annex III (Basic Counting Procedures), was used included in Instrução Normativa N. 62 (MAPA, 2003).

Experimental design and statistical analysis

The effects of acid concentration (X_1), storage time (X_2), and the number of daily stirrings of the ensiled mass (X_3) on pH (Y_1), protein content (Y_2), lipid oxidation (Y_3), oil production (Y_4), and mesophilic count (Y_5) in the ensiled mass were evaluated. RSM was used, following an incomplete 3^3 factorial design with three repetitions at the central point (Box & Behnken, 1960), as described in Table 1.

Statistical analysis was performed using the Statistica 7.0 software (Statsoft, 2004), and the choice of response surface model parameters was based on the significance level (0.05) of the independent variables.

Analysis of variance indicated the behavior of a system combining the independent variables (X) (X_1 = acid concentration; X_2 = storage time; X_3 = number of daily stirrings of the ensiled mass) and dependent variables or responses (Y). The responses were a function of the levels at which the independent variables were defined and combined in the statistical design.

The influence of the independent variables was observed through response surface graphs developed from the models. The adequacy of the models was evaluated based on the coefficient of determination (R^2).

Results and Discussion

Silage production

The ensiled mass, under the action of phosphoric and acetic acids, showed a color change from reddish to brown, with a pasty consistency. Acid incorporated into the ensiled mass replaced the main biochemical function of microorganisms, which produce lactic acid during fermentation.

Generally, lactic acid, produced by microorganisms, lowers the pH values of silage, causing protein hydrolysis (Oliveira et al., 2006). There was liquefaction of the ensiled mass during the first week of storage due to the action of endogenous enzymes from the chicken carcasses, enhanced by the acidic environment. This occurrence of liquefaction was also reported by Tatterson (1982) when working with fish silage at temperatures of 20 to 30°C.

Through response surface analysis, it was possible to demonstrate the influence of the studied variables in the production of acidic chicken silage, as well as to obtain precise values for these variables, maximizing the production process.

The model obtained by RSM to represent the effect of the independent variables (acid concentration, storage time, and number of daily stirrings) on the pH of the ensiled mass is: Y_1 =3.9– 0.58.X_1 +

$0.14.X_1^2 + 0.04.X_2 - 0.05.X_1.X_3$; where Y1 = pH of the ensiled mass (R^2 =0.97); X1 = acid concentration; X2 = storage time; X3 = number of daily stirrings; R^2 = coefficient of determination (Figure 1).

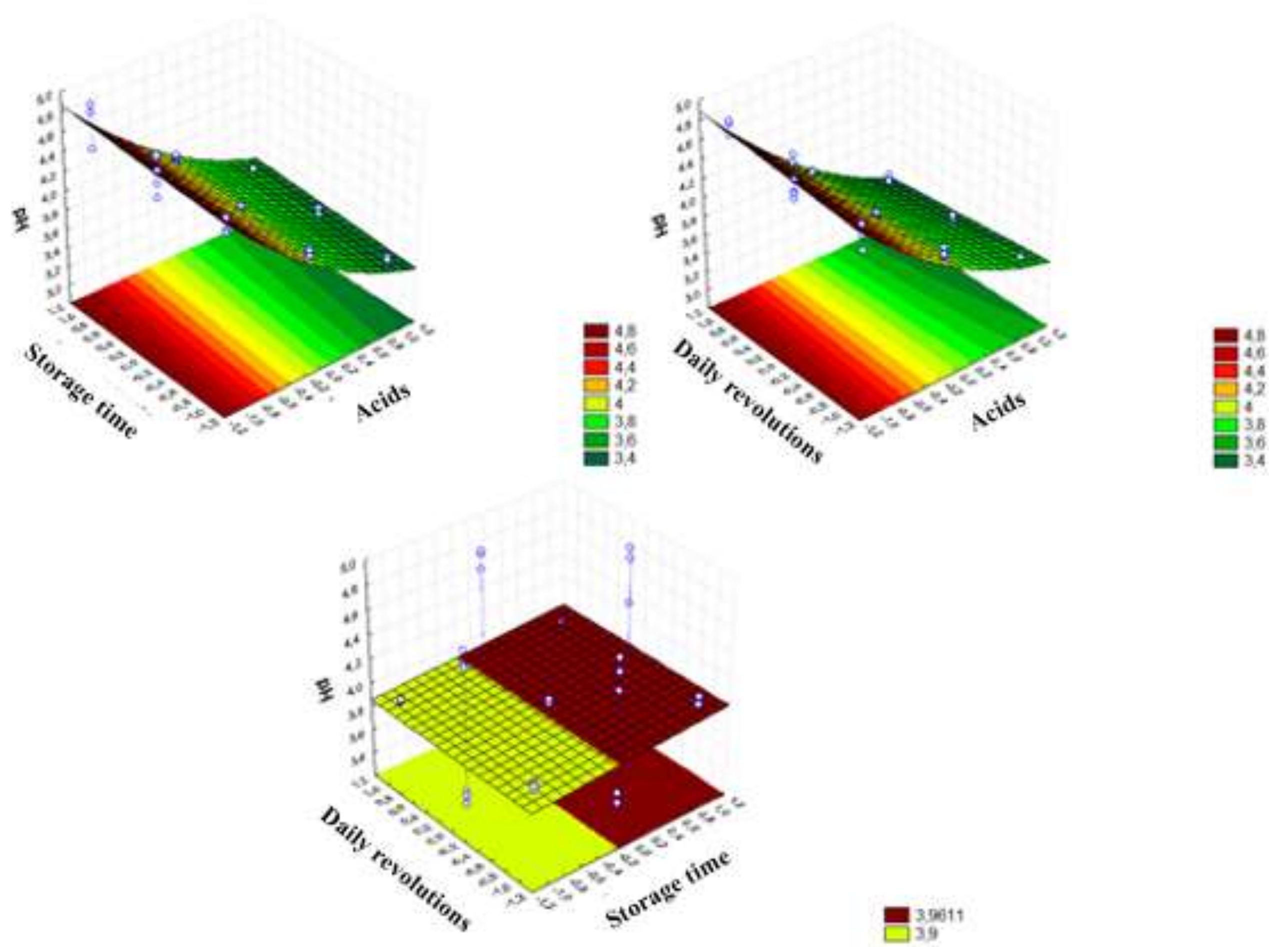

Figure 1. Response surface of the pH of acid silage from chicken carcasses as a function of acid concentration (phosphoric and acetic), storage time, and daily revolutions.

The acid concentration (X_1) had the greatest influence on the pH of the ensiled mass, with a negative linear effect, meaning that the higher the acid concentration, the lower the pH, and a positive quadratic effect, indicating a trend toward a minimum pH near the central point (Figure 1). These results may be due to the supply of hydrogen ions (protons, H^+) to the ensiled mass by the acids.

Physicochemical analyses

Table 2 shows the chemical compositions of the acidic silages of chicken carcasses, obtained from the addition of different concentrations of phosphoric and acetic acids.

Evaluating the physicochemical characteristics of acidic silage made from whole fish subjected to acidification with acetic acid, Pessoa et al. (2018) observed an average pH value of 4.0 from the first day of storage. Middleton and Ferket (2001a) also observed decreasing pH values as the concentration of phosphoric acid increased, ranging from 4.13% to 8.28%, in order to preserve chicken carcass silages.

Storage time (X_2) had a positive linear effect on pH over the storage period of the ensiled mass (Figure 1). Middleton et al. (2001b) reported similar observations regarding the pH variation of chicken waste silage, they observed an increase in pH from 4.2 to 4.75 after 45 days of storage.

There was no effect of the number of daily stirrings (X_3) on pH. However, the interaction between acid concentration (X_1) and the number of stirrings (X_3) had a negative effect, meaning that the pH of the mass decreased when higher acid concentrations (X_1) were combined with an increased number of stirrings (X_3). From a practical standpoint, if the goal is to lower the pH of the ensiled mass, it is important that, under higher acid concentrations, the mass is stirred more frequently. According to Oetterer (1994), after the addition of acids to the ensiled mass, an initial stirring of the mass is necessary, followed by occasional stirrings to ensure uniformity. This also influences the fermentation process, since microorganisms are sensitive to environmental changes (Guimarães et al., 2021), and it helps prevent the decay of regions that do not come into contact with the acid.

In evaluating the inclusion of ensiled chicken intestines and blood in tilapia feed, Diaz-Cachay et al. (2023) observed a pH value of 4.2 at 48 h of storage, similar to Batalha et al. (2017), who observed a pH value of 4.4 for acidic fish silages after 72 h of storage, allowing for the preservation of the silage and bacteriostatic activity.

Table 2
Average chemical composition (%) of acid silage from chicken carcasses, obtained from the addition of different concentrations of phosphoric and acetic acids

Treatments	MOI	CP[1]	EE[1]	Ca[1]	P[1]	MM[1]
1	65.62	55.35	31.55	2.03	7.86	10.84
2	64.14	48.61	32.07	2.02	7.02	13.93
3	65.35	53.72	35.01	3.03	7.51	9.78
4	64.59	50.07	28.49	2.69	8.01	14.26
5	63.25	46.14	35.15	2.45	7.74	10.51
6	63.86	48.36	30.19	2.80	8.28	14.27
7	64.68	50.59	29.86	3.81	9.00	12.05
8	65.11	52.14	30.40	2.59	9.21	16.36
9	64.79	47.91	32.88	2.58	9.18	13.80
10	64.68	52.60	31.87	2.20	5.61	13.08
11	66.97	56.88	30.63	2.88	8.19	13.93
12	66.38	57.62	30.93	2.84	7.80	14.44
13	66.22	57.51	29.22	2.24	5.54	15.07
14	66.64	58.44	31.93	2.10	5.64	14.56
15	67.21	56.30	30.37	2.15	5.44	14.61
Average	65.30±1.18	52.82±4.07	31.37±1.89	2.56±0.48	7.47±1.33	13.43±1.84

[1] on a dry basis; MOI = moisture; CP = crude protein; EE = ether extract; Ca = calcium; P = phosphorus; MM = mineral matter.

In Figures 1 and 2, the response surface and the observed and predicted pH values of chicken silage are shown, respectively. Both indicated that, when the acid concentration (X_1) is at the coded level 0, that is, 5% acids, the pH remains around 4. This pH value is the most suitable for the preparation and preservation of the silage.

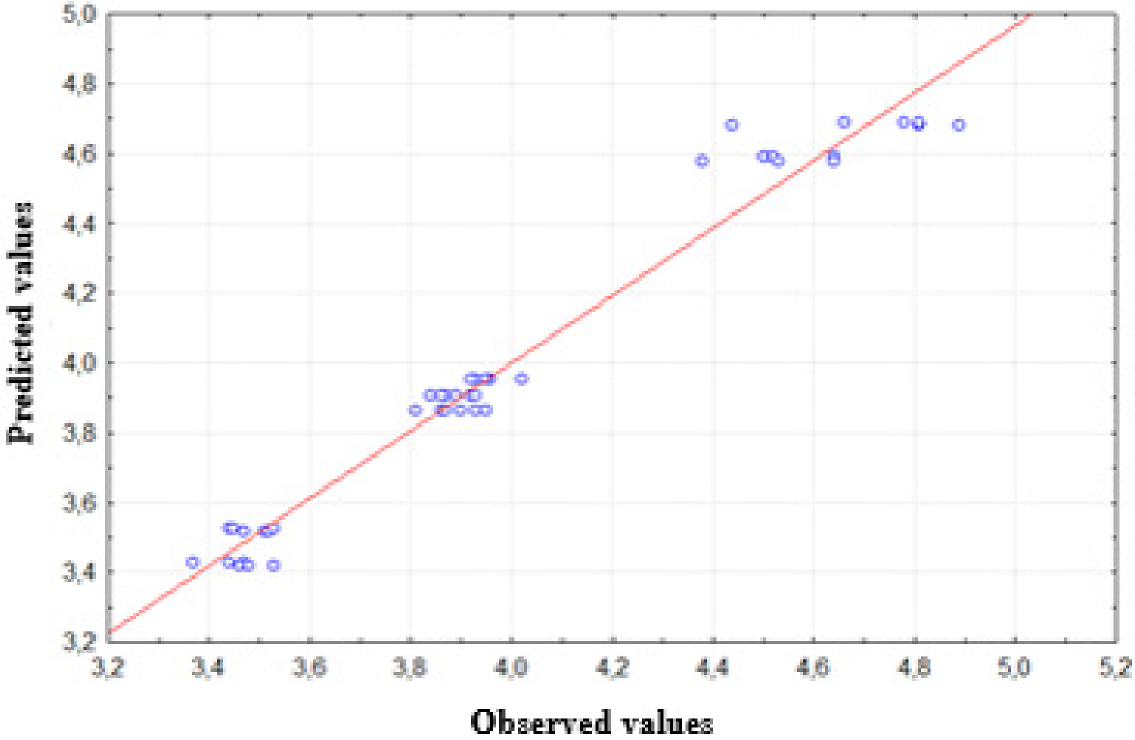

Figure 2. Observed and predicted values of the pH of acid silage from chicken carcasses, with X_3 (number of daily revolutions) fixed at level 0 and varying X_1 (acids, %) and X_2 (storage days).

The model used to represent the effect of the independent variables on the protein value of the ensiled mass is $Y_2 = 58.41 – 0.59.X_1 – 4.18.X_1^2 – 2.23.X_2^2 + 3.12.X_3 – 2.35.X_3^2 + 1.70.X_1.X_2$; where Y_2 = CP of the ensiled mass (R^2 =0.78); X_1 = acid concentration; X_2 = storage time; X_3 = number of daily stirrings; R^2 = coefficient of determination (Figure 3).

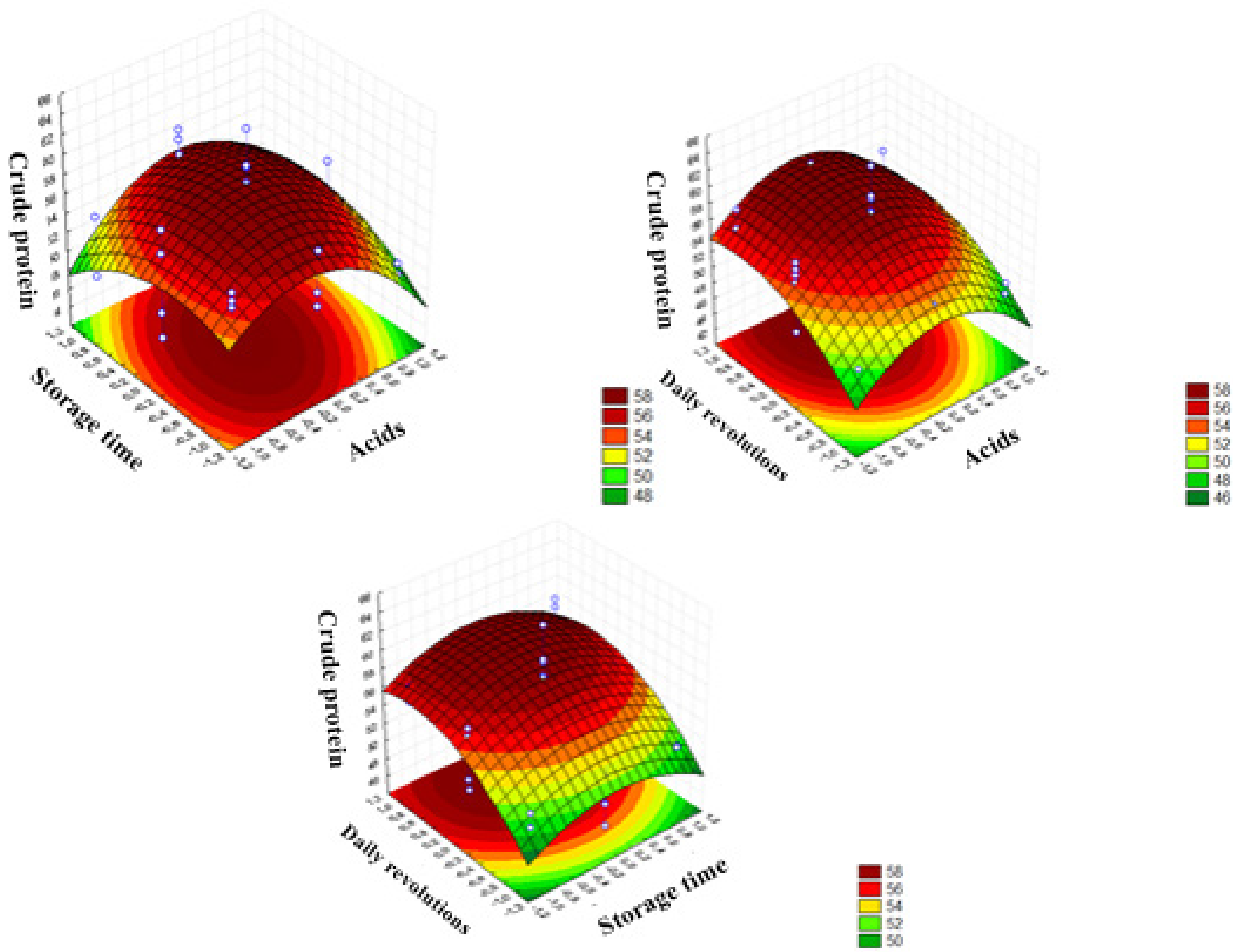

Figure 3. Response surface of the crude protein of acid silage from chicken carcasses as a function of acid concentration (phosphoric and acetic), storage time, and daily revolutions.

The acid concentration variable (X1) showed a negative linear effect, meaning that the higher the acid concentration used, the lower the CP content, and a negative quadratic effect, indicating a region of maximum protein value near the central point (Figure 3). There may have been an effect of dilution caused by the increase in acids in the ensiled mass, as well as the action of proteolytic enzymes favored by the pH reduction.

Similar results were observed by Borghesi et al. (2007) when evaluating acidic, biological, and enzymatic silages using by-products from fish processing and fish farming discards. Middleton and Ferket (2001a) observed that chicken silage prepared with phosphoric acid, with higher concentrations of these acids, promoted lower values of CP. The CP values found in silages with higher levels of phosphoric acid (6.90% and 8.28%) were similar to the

CP levels found in the lactic fermentation of chicken carcasses.

Storage time (X_2) had a negative quadratic effect, indicating a maximum protein value at the central point, that is, around 20 days of storage (Figure 3). According to Oetterer (1994), in acidic silage, the hydrolysis caused by the acid makes nitrogen more soluble. During ensiling, a portion of the protein nitrogen is converted into ammonia (Raghunath & McCurdy, 1987), which may be lost through volatilization, potentially justifying the decrease in protein content during prolonged storage.

The number of daily stirrings (X_3) had a positive linear effect, meaning that the greater the number of stirrings, the higher the protein content, and a negative quadratic effect, indicating a maximum point close to the central point. With the stirring of the ensiled mass, there is a better distribution of acids, enhancing hydrolysis, as observed by Oetterer (1994). There was an interaction between X_1 and X_2 (acid concentration and days of storage), presenting a positive effect, meaning that the CP value increased when both variables were raised.

The CP values found in chicken silage are close to the values found in chicken viscera meal, which ranges between 57.4% and 55.6% CP on a DM basis, as observed by Rostagno et al. (2024). The protein values of silage may vary according to the ensiling methodology and the composition of the raw material (Borghesi et al., 2007). Fagbenro and Faskin (1996) found values of 54.1% CP on a DM basis in silage made from chicken viscera with the addition of citric and propionic acids (4.5% and 0.5%, respectively). Borghesi et al. (2007) obtained CP levels of around 54.25% in acidic silages made from by-products of fish processing and fish discards.

Lower values were reported by Kherrati et al. (1998) from chicken by-products (viscera, feathers, head, and feet) ensiled with 15% molasses, finding CP values ranging from 30.5% to 41.9% in the silage. Cai and Sander (1995), combining concentrations of 5% to 8% corn flour and 5% and 8% dehydrated whey to compose chicken carcass silage, found CP values ranging from 41.5% to 49.2%.

The model used to represent the effect of the independent variables on the lipid oxidation value of the ensiled mass is $Y_3 = 0.022 - 0.003X_1 + 0.007X_1^2 - 0.009X_2 - 0.002X_3 + 0.007X_3^2$, where Y_3 = lipid oxidation of the ensiled mass ($R^2 = 0.66$); X_1 = acid concentration; X_2 = storage time; X_3 = number of daily stirrings; R^2 = coefficient of determination (Figure 4).

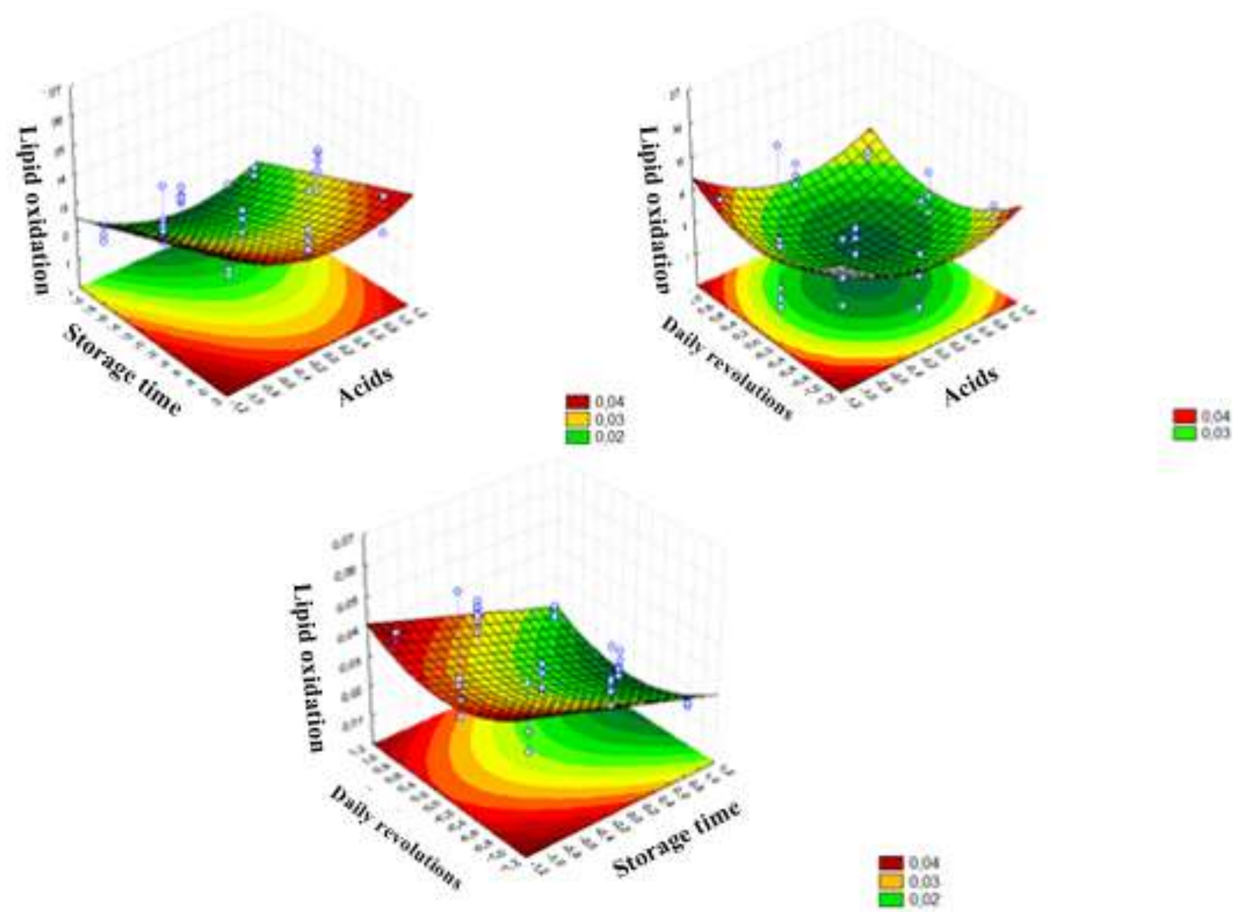

Figure 4. Response surface of lipid oxidation in acid silage from chicken carcasses as a function of acid concentration (phosphoric and acetic), storage time, and daily revolutions.

The acid concentration (X_1) showed a negative linear effect, meaning that the higher the acid concentration used, the lower the lipid oxidation of the ensiled mass, and a positive quadratic effect, indicating a tendency for minimal oxidation near the central point (Figure 4). Possibly, the acid acted as a primary antioxidant. Considering that the primary action of antioxidants is exerted owing to their ability to donate hydrogen atoms to free radicals formed. The storage time (X_2) exhibited a linear effect, meaning that the longer the storage period, the lower the lipid oxidation (Figure 4).

The number of daily stirrings (X_3) showed a negative linear effect. The greater the number of stirrings, the lower the lipid oxidation. However, the quadratic effect was positive, indicating a tendency for minimal oxidation near the central point (Figure 4).

Long storage times negatively affect the nutritional value and conservation efficiency of silage. Loss of nutritional value is related to darkening, caused by lipid reactions. Lipid oxidation alters the flavor, color, texture, and nutritional value (Kompiang, 1981), in addition to producing peroxides that can complex proteins through covalent bonds, which destroy amino acids such as tryptophan, oxidize methionine, and link lysine to compounds that render it unavailable (Diaz-Cachay et al., 2023).

In this study, there was a decrease in lipid oxidation over time. The values observed, being low and very close to each other, likely forced a tendency to decrease the lipid oxidation of the treatments. Raj et al. (2018) found low thiobarbituric acid values in fish silage (4.25 mg 100 mg⁻¹), considered the threshold of rancidity perception.

The model obtained to represent the effect of the independent variables on the oil extraction from the ensiled mass is represented by the following equation: $Y_4 = 4.27 + 0.653X_1 + 1.582X_1^2 - 1.554X_3 - 0.571X_1X_2 + 0.752X_1X_2^2 + 0.290X_1^2X_2 + 0.939X_1X_3 + 1.751X_1^2X_3 - 1.241X_2X_3$; where, Y_4 = oil extraction from the ensiled mass (R^2 =0.49); X_1 = acid concentration; X_2 = storage time; X_3 = number of daily stirrings; R^2 = coefficient of determination (Figure 5).

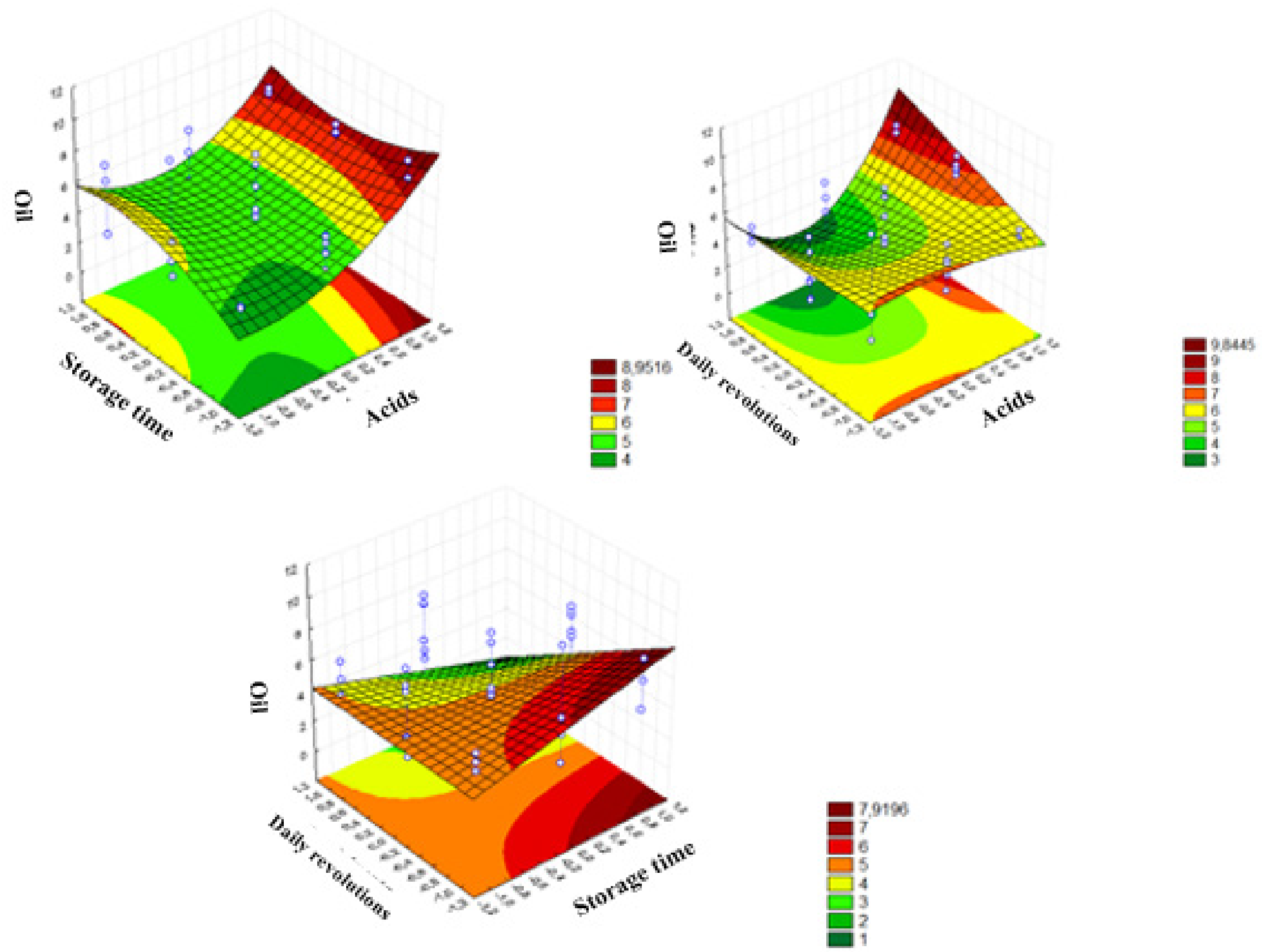

Figure 5. Response surface of oil extraction from acid silage of chicken carcasses as a function of acid concentration (phosphoric and acetic), storage time, and daily revolutions.

The acid concentration (X_1) showed a positive linear effect, meaning that the higher the acid concentration, the greater the oil extraction, along with a positive quadratic effect, indicating a tendency for minimal oil extraction near the central point (Figure 5).

The storage time (X_2) did not show a significant effect, and the number of daily stirrings (X_3) exhibited a negative linear effect, meaning that the greater the number of stirrings, the lower the oil extraction from the ensiled mass (Figure 5). As the number of daily stirrings increased, the oil was re-incorporated into the silage. In treatments with a higher number of stirrings, the silage appeared to have emulsified characteristics. Seibel and Souza-Soares (2003), analyzing silage from marine fish by-products, also observed the formation of emulsions in silage when the number of stirrings was increased.

Although the coefficient of determination was low, the response surface analysis indicated regions of minimal oil extraction values when the acid concentration (X_1) was close to the central point, that is, 5% acids. The regions of maximum oil extraction become less interesting, as the goal of silage is to generate a product that serves as an ingredient in animal feed rather than oil extraction.

Oil extraction could be directed toward biodiesel production. Gomes et al. (2000) produced biodiesel from inedible chicken oil generated in slaughterhouses of cooperatives in the western region of Paraná State, reporting a yield of 95%, which is considered satisfactory.

However, this study found that, to achieve a good oil yield, the acid concentration (X_1) must be high. This causes a decrease in the protein value of the silage and increases production costs. In contrast, another way to maximize oil production is to decrease the acid concentration (X_1) and the number of daily stirrings (X_3), which would also harm the quality of the produced silage.

No studies reported oil extraction during the process of obtaining silage from chicken carcasses. The values found in this study are close to those reported by Vidottti and Gonçalves (2006), who reported oil extraction values ranging from 6.77% to 8.65% in fish by-product silage.

Microbiological analyses

The model obtained to represent the effect of the independent variables on the mesophilic count of the ensiled mass is represented by the following equation: $Y_5 = 3.10 + 0.18.X_1 - 0.16.X_2 + 0.47.X_2^2 + 0.94.X_1.X_2 - 1.18.X_1.X_2^2 - 0.54X_1^2.X_2$; where, Y_5 = mesophilic count of the ensiled mass.

The model obtained to represent the effect of the independent variables on the mesophilic count of the ensiled mass is represented by the following equation: mesophilic count of the ensiled mass (R^2 =0.81); X_1 = acid concentration; X_2 = storage time; X_3 = number of daily revolutions; R^2 = coefficient of determination (Figure 6).

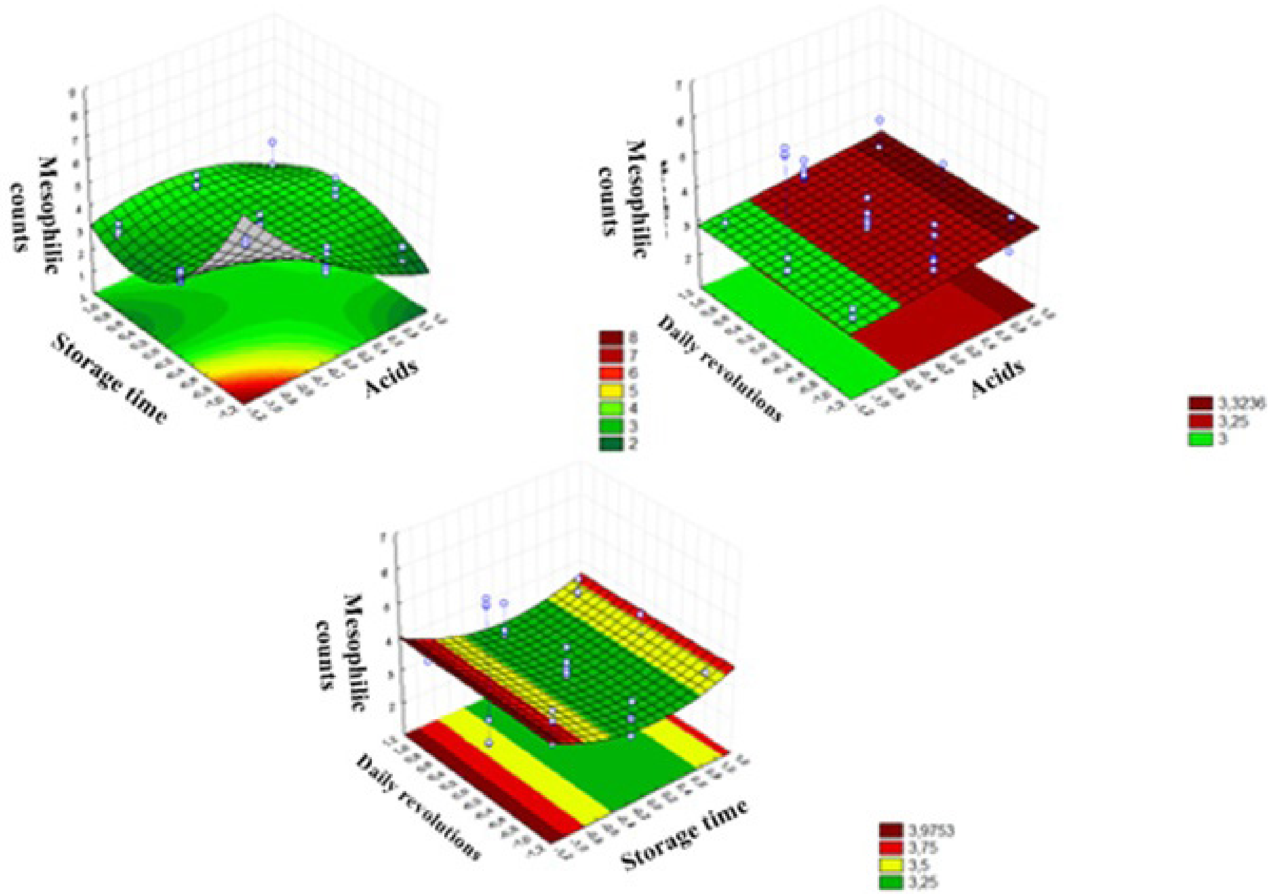

Figure 6. Response surface of mesophilic counts (in log) of acid silage from chicken carcasses as a function of acid concentration (phosphoric and acetic), storage time, and daily revolutions.

Among the evaluated variables, the number of daily revolutions (X_3) did not show a significant effect. The acid concentration (X_1) exhibited a positive linear effect, meaning that the higher the acid concentration, the greater the mesophilic count. However, there were interactions between the acid concentration (X_1) and storage time (X_2) variables, showing greater negative coefficients, which means that the mesophilic count decreased with increasing acid concentration (X_1) and storage time (X_2) (Equation 5 and Figure 6).

The storage time (X_2) showed a negative linear effect. The longer the storage time, the lower the mesophilic count, and a positive quadratic effect indicated a tendency for the mesophilic count to be minimal close to the central point (Figure 6).

In evaluating the mesophilic count, the objective was to find the point where there would be the lowest possible number of microorganisms. Therefore, concentrations of acids below 5% should be avoided, and the ensiled mass should be stored for more than 20 days (Figure 6).

Although there are differences in the mesophilic count of the silages among the different treatments, the MPN of coliforms at 45°C per g and the MPN of coliforms at 30°C per g were below 0.3 MPN g^{-1}. The National Health Surveillance Agency, through Resolution RDC No. 12, established that, in cooked meat foods, the acceptable limit is up to 10^3 MPN/g. The contamination in the raw material was 496,000 MPN/g of coliforms at 45°C, and 750,000 MPN/g of coliforms at 30°C. Other researchers working with fish waste silage (Oliveira et al., 2006; Pessoa et al., 2018) and chicken carcass silage (Cai & Sander, 1994; Kherrati et al., 1998; Middleton & Ferket, 2001a; Shaw et al., 1998) also verified the efficiency of the ensiling process in eliminating pathogens. The reduction of contamination after acid treatments demonstrates the effectiveness of eliminating pathogens and making the product safe.

A patent was granted on July 14, 2020, for this work under registration number PI000215 at the National Institute of Industrial Property.

Conclusion

RSM allowed for the optimization of the independent variables (acid concentration, storage time, and number of daily revolutions) in the process of preparing chicken carcass silage. The recommended acid concentration is 2% phosphoric acid and 3% acetic acid, for a minimum period of 20 days, with stirring once a day, which provides better uniformity, allowing hydrolysis to occur homogeneously throughout the ensiled mass.

References

Association of Official Analytical Chemists (2016). *Official methods of analytical of the association of official analytical of chemists* (20nd ed.). AOAC.

Batalha, O. S., Alfaia, S. S., Cruz, F. G. G., Jesus, R. S., Rufino, J. P. F., & Costa, V. R. (2017). Digestibility and physico-chemical characteristics of acid silage meal made of pirarucu waste in diets for commercial laying hens. *Acta Scientiarum. Animal Sciences, 39*(3), 251-257. doi: 10.4025/actascianimsci.v39i3.35112

Beerli, E. L., Beerli, K. M. C., & Logato, P. V. R. (2004). Silagem ácida de resíduos de truta (Oncorhynchus mykiss), com a utilização de ácido muriático. *Ciência e Agrotecnologia, 28*(1), 195-198. doi: 10.1590/S1413-70542004000100026

Belc, N., Mustatea, G., Apostol, L., Iorga, S., Vlăduț, V. N., & Mosoiu, C. (2019). Cereal supply chain waste in the context of circular economy. In: E3S Web of Conferences, Târgovişte, Romênia, *8th International Conference on Thermal Equipment, Renewable Energy and Rural Development (TE-RE-RD 2019),* 112, 03031. doi: 10.1051/e3sconf/201911203031

Borghesi, R., Arruda, L. F., & Oetterer, M. (2007). A silagem de pescado na alimentação de organismos aquáticos. *Boletim do Ceppa, 25*(2), 329-339.

Blake, J. P. (2004). Methods and technologies for handling mortality losses. *World's Poultry Science Journal, 60*(4), 489-499.

Borrajo, P., Pateiro, M., Barba, F. J., Mora, L., Franco, D., Toldrá, F., & Lorenzo, M. J.

(2019). Antioxidant and antimicrobial activity of peptides extracted from meat by-products: a review. *Food Analytical Methods, 12*(11), 2401-2415. doi: 10.1007/s12161-019-01595-4

Box, G. E. P., & Behnken, D. W. (1960). Some new three level designs for the study of quantitative variables. *Technometrics, 2*(4), 455-475.

Cai, T., & Sander, J. E. (1995). Fermentation mixture formulation and preservation of poultry carcasses. *Journal of Applied Poultry Research, 4*(1), 88-93. doi: 10.1093/japr/4.1.88

Camilios, D., Neto, Buzato, J. B., Celligoi, M. A. P. C., & Oliveira, M. R. (2005). Otimização da produção de etanol por Zymomonas mobilis na fermentação do melaço de cana-de-Açúcar. *Semina: Ciências Exatas e Tecnológica, 26*(1), 17-22. doi: 10.5433/1679-0375.2005v26n1p17

Chakka, A. K., Elias, M., Jini, R., Sakhare, P. Z., & Bhaskar, N. (2015) In-vitro antioxidant and antibacterial properties of fermentatively and enzymatically prepared chicken liver protein hydrolysates. *Journal Food Science and Technology, 52*(12),8059-8067. doi: 10.1007/s13197-015-1920-2

Díaz-Cachay, C., Gamero-Collado, B., Alvarez-Verde, C., Llontop-Vélez, C. & Zambrano-Cabanillas, A. W. (2023). Efecto de ensilados de sangre e intestinos de pollo, como sustitutos parciales de la harina de pescado, en el crecimiento de alevinos de tilapia Oreochromis niloticus (Linnaeus, 1758). *Revista de Investigaciones Veterinaria del Perú, 34*(5), e24624. doi.org/10.15381/rivep.v34i5.24624

Diniz, F., & Martin, A. M. (1996). Use of response surface methodology to describe the combined effects of pH, temperature and E/S ratio on the hydrolysis of dogfish (*Squalus acanthias*) muscle. *International Journal of Food Science and technology, 31*(5), 419-426. doi: 10.1046/j.1365-2621.1996.00351.x

Eissa, A., Yusuf, M., Karamat, N. A., Badran, M., Dessouki, A. A., Ismail, G., Ford, H., & Abdelatty, A. (2021). Effect of poultry offal silage with or without betaine supplementation on growth performance, intestinal morphometry, spleen histomorphology of Nile tilapia (*Oreochromis niloticus*) fingerlings. *Journal of Animal Physiology and Animal Nutrition, 106*(11). doi: 10.1111/jpn.13655

Fagbenro, A., & Fasakin, E. (1996). Citric-acid-ensiled poultry viscera's as protein supplement for catfish (*Clarias gariepinus*). *Bioresource Technology, 58*(1), 13-16. doi: 10.1016/S0960-8524(96)00081-8

Galali, Y., Omar, Z. A., & Sajadi, S. M. (2020). Biologically active components in by-products of food processing. *Food Science & Nutrition, 8*(7), 3004-3022. doi: 10.1002/fsn3.1665

Gao, Y., Lo, K. V., & Liao, P. H. (1992). Utilization of salmon farm mortalities: fish silage. *Bioresource Technology, 41*(2), 123-127. doi: 10.1016/0960-8524(92)90181-V

Gomes, L. F. S., Souza, S. N. M., Bariccatti, R. A., & Souza, J. (2000). Potencial de produção de biosiesel a partir do óleo de frango nas cooperativas do oeste do Paraná. *Varia Scientia, 4*(8), 141-149.

Guimarães, C. C., Maciel, I. V., Silva, A. F., Lopes, A. F., Carpio, K. C. R., & Silva, A. J. I. (2021). Aspectos biotecnológicos da silagem biológica de resíduos do Tambaqui. *Revista em Agronegócio e Meio Ambiente, 14*(1), 205-215. doi: 10.17765/2176-9168.2021v14n1e006861

Hisano, H., & Borghesi, R. (2011). Elaboração de silagem ácida de vísceras de surubim (*Pseudoplatyustoma* sp.). (Circular Técnica, 18). Embrapa, Dourados (MS), Brazil.

Hubbard, L. E., Givens, C. E., Griffin, D. W., Iwanowicz, L. R., Meyer, M. T., & Kolpin, D. W. (2020). Poultry litter as potential source of pathogens and other contaminants in groundwater and surface water proximal to large-scale confined poultry feeding operations. *The Science of the Total Environment, 735,* 139459. doi: 10.1016/j.scitotenv.2020.139459

Instrução Normativa no 62, de 26 de Agosto. *Diário Oficial da União,* Brasília, 26 de agosto de 2003. Seção 1. Ministério da Agricultura, Pecuária e Abastecimento_MAPA. Secretaria de Defesa Agropecuária - DISPOA.

Kannah, R. Y., Merrylin, J., Devi, T. P., Kavitha, S., Sivashanmungam, P., Kumar, G., & Banu, J. R. (2020). Food waste valorization: biofuels and value added product recovery. *Bioresource Technology Reports, 11,* 100524. doi: 10.1016/j.biteb.2020.100524

Kherrati, B., Faid, M., Elyachioui, M., & Wahmane, A. (1998). Process for recycling slaughterhouses waste and by-products by fermentation. *Bioresource Techology, 63*(1), 75-79. doi: 10.1016/S0960-8524(97)00 081-3

Kompiang, I. P. (1981). Fish silage: its prospect and future in Indonesia. *Indonesian Agriculture Resource & Development Journal, 3*(1), 9-12.

McGauran, T., Dunne, N., Smyth, B. M., & Cunningham, E. (2021). Feasibility of the use of poultry waste as polymer additives and implications for energy, cost and carbon. *Journal of Cleaner Production, 291* (125948). doi: 10.1016/j.jclepro.2021.125948

Middleton, T. F., & Ferket, P. R. (2001a). Effect of level of acidification by phosphoric acid, storage temperature, and length of storage on the chemical and biological stability of ground poultry mortality carcasses. *Poultry Science, 80*(8), 1144-1153. doi: 10.1093/ps/80.8.1144

Middleton, T. F., Ferket, P. R., Boyd, L. C., Daniels, H. V., & Gallagher, M. L. (2001b). An evaluation of co-extruded poultry silage and culled jewel sweet potatoes as a feed ingredient for hybrid tilapia (*Orecohromis niloticus* x *O. mossambicus*). *Aquaculture, 198*(3-4), 269-280. doi: 10.1016/S0044-8486(00)00601-3

Malavolta, E., Vitti, G. C., & Oliveira, S. D. (1997). *Avaliação do estado nutricional das plantas: princípios e aplicações* (2a ed.). Potafos.

Oetterer, M. (1994). Produção de silagem a partir da biomassa residual de pescado. *Alimentos e Nutrição, 5*(1), 119-134.

Oliveira, M. M., Pimenta, M. E. S. G., Camargo, A. C. S., Fiorini, J. E., & Pimenta, C. J. (2006). Silagem de resíduos da filetagem de tilápia do Nilo (*Oreochromis niloticus*), com ácido fórmico - análise bromatológica, físico-química e miológica. *Ciência e Agrotecnologia, 30*(6), 1218-1223. doi: 10.1590/S1413-70542006000600027

Pessoa, M. S., Abrão, F. O., Duarte, E. R., Camargo, A. C., & Faria, D. E., Fº. (2018). Physical-chemical and microbiological characteristics of acid silage of fi sh subjected to two processes of acidification and different storage periods. *Zootecnia Tropical, 36*(3-4), 97-105.

Rachmawati, D., & Samidjan, I. (2019). The effects of chicken feather silage substitution for fish meal in the diet on growth of saline tilapia fingerlings (*Oreochromis niloticus*). *Proceedings of the International Conference on Tropical and Coastal Region Eco Development,* Semarang, Indonésia, 246, 012015. doi: 10.1088/1755-1315/246/1/012015.

Raj, R., Raju, C. V., Lakshmisha, I. P., & Jag, P. (2018). Nutritional and biochemical properties of fish silage prepared as an ingredient in poultry feed. *International Journal of Current Microbiology and Applied Science, 7*(5), 423-428. doi: 10.20546/ijcmas.2018.705.054

Raghunath, M. R., & McCurdy, A. R. (1987). Autolysis-resistant sediment in fish silage. *Biological Wastes, 20*(3), 227-239. doi: 10.1016/0269-7483(87)90157-1

Rostagno, H. S., Albino, L. F. T., Calderano, A. A., Hannas, M. I., Sakomura, N. K., Perazzo, F. G., Rocha, G. C., Saraiva, A., Abreu, M. L. T., Genova, J. L., & Tavernari, F. C. (2024). *Tabelas brasileiras de aves e suínos: composição de alimentos e exigências nutricionais* (5a ed.). Suprema.

Seibel, N. F., & Souza-Soares, L. A. (2003). Produção de silagem química com resíduos de pescado marinho. *Brazilian Journal Food Technology, 6*(2), 333-337.

Shaw, D. M., Narasimha Rao, N. D., & Mahendrakar, N. S. (1998). Rapid fermentation for ensiling of poultry intestines. *Bioresource Technology, 65*(3), 247-249. doi: 10.1016/S0960-8524(98)80001-1

Sinnhuber, R. O., & Yu, T. C. (1977). The 2-thiobarbituric acid reaction, an objective measure of the oxidative deterioration occurring in fats and oils. *Journal of Japan Oil Chemists Society, 26*(5), 259-267.

Tatterson, I. N. (1982). Fish silage preparation, properties and uses. *Animal Feed Science and Technology, 7*(4), 153-159. doi: 10.1016/0377-8401(82)90050-5

Vidotti, R. M., & Gonçalves, G. S. (2006). Produção e caracterização de silagem, farinha e óleo de tilápia e sua utilização na alimentação animal. Instituto de Pesca - APTA-SAA. http://www.pesca.sp.gov.br

Vidotti, R. M., Viegas, E. M. M., & Carneiro, D. J. (2003). Amino acid composition of processed fish silage using different raw materials. *Animal Feed Science and Technology, 105*(1-4), 199-204. doi: 10.1016/S0377-8401(03)00056-7

Vidotti, R. M. & Gonçalves, G. S. (2006). Produção e caracterização de silagem, farinha e óleo de tilapia e sua utilização na alimentação animal. *Instituto de pesca - APTA-SAA.* http://www.pesca.sp.gov.br

Voběrková, S., Maxianová, A., Schlosserová, N., Adamcová, D., Vršanská, M., Richtera, L., Gagić, M., Zloch, J., & Vaverková, M. D. (2020). Food waste composting. Is it really so simple as stated in scientific literature? A case study. *The Science of the Total Environment, 723,* 138202. doi: 10.1016/j.scitotenv.2020.138202