

# Industrial and culinary practice effects on biologically active polyamines level in turkey meat

# Moein Bashiry<sup>1</sup>, Hedayat Hosseini<sup>2</sup>\*, Abdorreza Mohammadi<sup>2</sup>\*, Ehsan Sadeghi<sup>1</sup>, Nader Karimian-Khosroshahi<sup>3</sup>, Francisco J. Barba<sup>4</sup>, Amin Mousavi Khaneghah<sup>5</sup>

<sup>1</sup>Department of Food Science and Technology, School of Nutritional Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran; <sup>2</sup>Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences & Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran; <sup>3</sup>Food and Beverage Control Department, Iran Food and Drug Administration, Tehran, Iran; <sup>4</sup>Nutrition and Food Science Area, Preventive Medicine and Public Health, Food Science, Toxicology and Forensic Medicine Department, Faculty of Pharmacy, Universitat de València, Avda.Vicent Andrés Estellés, s/n, 46100 Burjassot, València, Spain; <sup>5</sup>Department of Food Science, Faculty of Food Engineering, University of Campinas, Campinas, SP – Brazil

\***Corresponding authors:** Hedayat Hosseini, Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences & Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: hedayat@sbmu.ac.ir and Amin Mousavi Khaneghah, Department of Food Science, Faculty of Food Engineering, University of Campinas, Campinas, SP – Brazil. Email: mousavi@unicamp.br

Submitted: 19 June 2020; Accepted: 21 April 2021; Published: 26 May 2021 © 2021 Codon Publications





# Abstract

Polyamines, including putrescine, spermidine, and spermine, are biological compounds present in nearly all food items. Their desirable physiological effects include cell division and growth. Hence, are undesirable in the diet of patients with tumor. This study aimed to assess the impact of curing agents (sodium chloride (0–2 g), sodium nitrite (0–200 ppm), sodium polyphosphate (0–0.5 g), and ascorbic acid (0–500 ppm)), cooking (frying (180°C), and boiling (100°C)) on polyamine contents in turkey breast meat using response surface methodology based on central composite design and dispersive liquid-liquid microextraction. Postprocessing changes were investigated using a high-performance liquid chromatography equipped with an ultraviolet detector. Study outcomes showed the presence of sodium chloride, sodium nitrite, and sodium polyphosphate in turkey meat reduced the putrescine and spermine content significantly (P < 0.0001). The addition of ascorbic acid as a curing agent slightly increased the concentration of polyamines, while no significant linear effects were associated with the thermal processes. The study observed that curing agents like sodium chloride, sodium nitrite, sodium polyphosphate, and ascorbic acid at 2 g, 200 ppm, 0.5 g, and 382 ppm, respectively, in frying mode minimized spermine and putrescine content with more than 96% desirability. In conclusion, curing additives and cooking are promising procedures for polyamine reduction in turkey breast meat.

Keywords: boiling; curing agents; frying; polyamines; RSM

# Introduction

Polyamines (PAs) are nitrogenous compounds that naturally exist in food items like meat, fish, cheese, and wine (Kalač, 2009; 2014a). The primary biogenic PAs include putrescine (PUT), spermidine (SPD), and spermine (SPM). These are endogenously synthesized by microbial activities, produced by microbiota, or taken in diet (El Adab *et al.*, 2020; Kamani *et al.*, 2015; Keşkekoğlu and Üren, 2013). Chromatographic methods like gas or liquid chromatography detect PAs. However, the most common analytical method that detect PAs is high-performance liquid chromatography (HPLC; Xu *et al.*, 2018). The physiological roles of PAs are gathered and reviewed in many scientific papers and books (Dandrifosse, 2009; Kalač, 2009; Muñoz-Esparza *et al.*, 2019). Accordingly, cell growth and differentiation are one of the PAs biological roles in the human body. However, the ingestion of these compounds can accelerate tumors and cancer cell growth (Kalač, 2014a). Therefore, the decrease PA concentrations in food products, particularly in meat and meat products, is crucial. Hence, experts suggest reduced PAs in the daily diet during cancer therapy. Intake of dietary PAs is desirable for accelerating the healing of wounds or burns (Kalač, 2014b).

Meat is a widely consumed food undergoing different technical processes (curing, freezing, cooking, and packaging) to make it digestible and safe (Mejri *et al.*, 2017). Cooking is a basic process commonly used before consumption and includes of thermal processes like boiling, frying, stewing, and grilling that influence organoleptic properties and make meat safer (Naseri *et al.*, 2010; Roseiro *et al.*, 2017b). On the other hand, curing is a usual practice applied in meat industries to improve the quality of products. In this regard, sodium chloride, nitrite, phosphate, and ascorbic acid are generally used to increase shelf life and meat quality. These additives influence microbial load, pH, lipid oxidation, water holding capacity, texture, and flavor (Kilic *et al.*, 2018).

Methods like the incorporation of additives in food formulations reduce the PAs concentration in food. Sodium chloride has a dramatic influence on biogenic amines and PAs content (Roseiro *et al.*, 2017a). Sodium nitrite, sodium nitrate, and other additives used in meat as preservatives, show destructive effects on microbial load and PAs (Hazar *et al.*, 2017). Other methods like thermal processes or packaging (vacuumed and modified) have a strong effect on food compositions (Pilevar *et al.*, 2019) and showed degradation effects on PAs in foods (Dadáková *et al.*, 2012; Kalač, 2014b). A previous study reported that all curing methods may influence PAs content (Triki *et al.*, 2018).

In this regard, many previous studies investigated the effects of various treatments on the PAs content of different meats (Dadáková *et al.*, 2012; Haddad *et al.*, 2018; Zhang *et al.*, 2015). The study by Kozová *et al.* (2009) evaluated the impact of thermal processing and packaging techniques for chicken meat and reported a significant decrease in PAs after frying (180°C), grilling (215°C), roasting (180–190°C), vacuum packaging, and modified atmosphere package (MAP). Another study by Hazar *et al.* (2017) assessed the changes of histamine, PUT, tyramine, tryptamine, cadaverine, SPD, and SPM concentrations in pastirma (a Turkish meat product) with different curing additives and duration. But to date, minimal researchers have studied the PAs concentration,

effects of additives, and thermal processes in turkey meat as its consumption has only increased in the recent years.

Among different food products, meat and meat products of animal origin are considered the major dietary source of PAs, especially SPM (Handa *et al.*, 2018). On the other hand, over the past decades, the consumption of poultry and processed poultry products has significantly increased (Hrynets *et al.*, 2011). Among the poultry, turkey meat is recommended by many nutritionists because of its moderate low-fat content, relatively higher polyunsaturated fatty acids, high levels of vitamin B, and low cholesterol (Amirkhanov *et al.*, 2017; Taheri *et al.*, 2018).

Response surface methodology (RSM) is a time-saving, valuable, and cost-effective tool that it decreases the number of experiments in a scientific mechanism (Araújo and de Aquino Santana, 2018; Pandiselvam *et al.*, 2019). The optimization of variables or design of a suitable predictive model can be easily obtained by using RSM (Shameena Beegum *et al.*, 2019; Srikanth *et al.*, 2020Srinivas *et al.*, 2020). Central composite design (CCD) is the commonly applied design to evaluate research variables and is also used in this study (Mokhtarian *et al.*, 2014; Sagarika *et al.*, 2018).

To the best of our knowledge, no study has reported the impact of curing and thermal processes on PAs content in turkey meat. Hence, the current study aimed to investigate the effects of different concentrations of curing agents (sodium chloride, sodium nitrite, sodium polyphosphate, and ascorbic acid) and the thermal processes (boiling and frying) on PA content in turkey breast meat. The study also optimized the curing agents' concentration and designed a mathematical model to predict responses by RSM.

# **Materials and Methods**

# **Chemical reagents**

SPM tetrahydrochloride, SPD trihydrochloride, PUT dihydrochloride (as standards), sodium nitrite, sodium tripolyphosphate, and dansyl chloride (derivative reagent) were purchased from Sigma-Aldrich (Louis, MO, USA). Analytical grade acetone, acetonitrile, water, and methanol, were obtained from Dae Jung (Busan, South Korea). Hydrochloric acid (37% w/w), perchloric acid, sodium hydroxide (NaOH), 1-octanol, ammonia, and sodium chloride (NaCl) were supplied by Merck (Darmstadt, Germany). Carrez solutions containing potassium hexaferrocyanide (Carrez solution I) and zinc acetate (Carrez solution II) were provided by Panreac Química SLU, Barcelona, Spain. Ascorbic acid was purchased from Dorshimi Marjan (Tehran, Iran). Carrez solutions I and II were prepared according to a previously described method (Ramezani *et al.*, 2015). Stock and standard working solutions, methodology for HPLC and microwave apparatus, and the conditions used for both techniques were previously established (Bashiry *et al.*, 2016).

#### Procedure

The freshly slaughtered turkey meat was purchased from the local market of Tehran, Iran, and was immediately transferred to the laboratory in cooled and sterile container (0°C). The meat samples of  $8 \times 8 \times 1$  cm and with a mean weight of  $50 \pm 2$  g were cubed out from the breast part using a kitchen knife. Later were immersed for 30 min in the curing solution containing defined amounts of Sodium chloride, sodium nitrite, sodium polyphosphate and ascorbic acid (Appendix A). and were refrigerated at 4 °C for 6 hours in glass containers (Busboom, 2003). The cured samples were divided into two parts. One portion was boiled in water (100°C) for 30 min, and another was fried in canola oil for 10 min, 5 min for each side of the sample, at 180°C (Kozová *et al.*, 2009) and cooled to room temperature.

#### PAs analysis

To determine the PA content of the prepared samples, acid extraction was carried out. Cured and cooked samples were minced and one gram of it was weighed and mixed with 8 mL of perchloric acid. This sample was microwaved (MDS-10 Sineo, Shanghai, China) at 500 MHz for 2 minutes to increase the velocity and efficiency of extraction. Later, the mixture was cooled, and mixed with 2 mL of Carrez solution (one mL I and one mL II) and centrifuged at 1252×g for 10 min at room temperature.

Next, the pH of the centrifuged solution was adjusted to 11 by adding NaOH. Later 1 mL of dansyl chloride was added to the tube, shaken vigorously, and incubated in a dark place at 40°C for 1 hour (shook occasionally) for effective processing (derivatization step). Finally, 250  $\mu$ L of ammonia was added to the tube to remove excess dansyl chloride, and, dispersive liquid-liquid microextraction (DLLME) was carried out by a previously reported method (Bashiry *et al.*, 2016).

#### DLLME and HPLC analysis

Briefly, 510  $\mu$ L of the extract solution (acetonitrile 450  $\mu$ L and 1-octanol 60  $\mu$ L) and 2 g of sodium chloride was added to the prepared samples (previous section) and shaken vigorously. Then, the samples were centrifuged (1252 ×g/10 min/room temperature). Twenty microliters

of the supernatant were collected and injected into an HPLC instrument (Cecil CE-4900, Cambridge, England) with the ultraviolet-Visible detector in duplicates. Working conditions of the HPLC: flow rate of the mobile phase (acetonitrile and water) was 0.8 mL/min, and the detector was set up on 254 nm. Moreover, the separation of the analytes was carried out using an octadecyl-silica column (250 mm  $\times$  4 i.d.  $\times$  5 µm) according to the method used by Bashiry *et al.* (2016).

#### Method validation

The calibration curves of PAs showed good linearity for all the PAs ( $R^2 > 0.98$ ) in the concentration range of 20-200 ng/g. To estimate the repeatability, the relative standard deviation (RSD) was evaluated by assessing peak areas from seven extractions of one turkey meat sample, which ranged from 6.72% to 7.30% for all PAs. Enrichment factor, representing the ratio of the final concentration of the analyte in the organic solvent to its original concentration, was 190, 210, and 305 for PUT, SPD, and SPM, respectively. The recovery of the DLLME procedure was determined by comparing with amounts of PAs added to the turkey breast meat sample and the residual concentrations after performing the procedure were 95%, 97.6%, and 105% for PUT, SPD, and SPM, respectively. The limits of detection for the three PAs (based on signal-to-noise ratios of three) under the optimum conditions were between 0.24 and 0.42 ng/g, and limits of quantification (based on signal-to-noise ratios of 10) were in the range 0.8-1.4 ng/g.

#### Data analysis

RSM based on CCD was applied to investigate the optimal level of sodium chloride, sodium nitrite, sodium polyphosphate, and ascorbic acid, to detect the effects of heat treatment (independent variables) on PAs. CCD in this design consisted of 60 runs for four numerical and one categorical variable. The curing agents (selected as numerical variables) were studied in five levels and two cooking types, selected as categorical variable are summarized in Table 1 to investigate linear effects and detect the optimal concentration to minimize PAs level in turkey meat. The PUT and SPM levels were the dependent variables, and P < 0.05 showed significant effects. Design Expert 11.0.3 software (Stat-Ease Inc., Minneapolis, MN, USA) was used for Data and regression analysis.

# **Results and Discussion**

Four common curing agents besides cooking procedures (boiling and frying) influencing PAs content were analyzed by RSM. Furthermore, the optimal concentrations of curing agents were also calculated for minimizing PAs

Independent variables	Unit	Symbol	Coded levels				
			-α	-1	0	+1	+α
Sodium chloride	g	А	0	0.5	1	1.5	2
Sodium nitrite	ppm	В	0	50	100	150	200
Sodium polyphosphate	g	С	0	0.13	0.25	0.38	0.5
Ascorbic acid	ppm	D	0	125	250	375	500
Cooking type	-	Е	Bo	biling		Frying	

Table 1. Levels of independent variables in Central Composite Design (CCD) design.

in turkey breast meat samples. The selection of variables was based on industrial practices and culinary treatments. Meat curing is a practice applied widely in food companies to improve the shelf life of the meat (Sebranek and Bacus, 2007). Curing additives like sodium chloride aids in digestion, nitrite influences both taste, color, and microbial load of product, and ascorbic acid helps to accelerate the curing process (Bakhtiary *et al.*, 2016; Posthuma *et al.*, 2018; Vidal *et al.*, 2019). On the other hand, domestic thermal techniques like boiling and frying influence biogenic amines and PAs in the meat matrix (Kalač, 2014a).

#### Analysis of parameters

To evaluate the effects of each independent variable on PAs (SPM and PUT), the design expert software suggested an appropriate mathematical model. This method was not capable enough to detect SPD responses.

# SPM analysis

According to the CCD results, the 2FI model was chosen. The Box-Cox plot, which helps determine the most appropriate transformation, suggested power transformation for a better model to predict responses. Therefore, data were transformed by the power function (Lambda: 0.87, Constant: 0.338), and the model was re-proposed. Equation (1) was used to predict the response for the given levels of each variable in both boiling and frying mode. This equation is pivotal as it can identify the relative influence of factors by comparing the coefficient of factors.

$$(\text{SPM} + 0.34)^{0.87} = +20.96 - 3.08 \text{ NaCl} - 0.06 \text{ Ni} - 7.74 pp + 0.0001 AA + 0.83 \text{ NaCl*pp} + 0.005 AA*pp (1)$$

where NaCl, Ni, pp, and AA are symbols showing concentrations of sodium chloride, sodium nitrite, sodium polyphosphate, and ascorbic acid, respectively. Table 2 shows the analysis of variance (ANOVA) for the curing process, showing the impact of the variables on the response (SPM level). Accordingly, any change in curing conditions has led to a significant (P < 0.0001) change of SPM content in meat samples.

ANOVA analysis showed that the 2FI model was significant (F = 4739.27). Additionally, lack of fit indicated no significant relation with a pure error. The coefficient determination obtained from ANOVA exhibited a strong relationship between the raw data and the proposed model  $(R^2 = 0.9984)$ . This tool provides an index of how wellobserved results are replicated by the model and estimates variation in the data calculated by the model. This proposed model explained 99.84% of the variation in the responses, and the 2FI model was compatible with the experimental data. The adjusted R<sup>2</sup> value recorded of 0.9982, represents the potential of the model to predict response. It is worth noting that the more the adjusted  $R^2$  values are closer to 1, the better is the model's predictability response. Finally, adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. Adequate precision in this study model was 277.959 indicating an sufficient signal.

Table 2 illustrates all independent variables except D term have a significant linear influence on SPM content (P < 0.0001); significant linear effects also illustrated in perturbation graph (Figure 1), in which destructive effect of sodium chloride, sodium nitrite, and sodium polyphosphate on SPM is clear. Ascorbic acid was found to be effective in a mild increase of SPM content in turkey breast meat.

# PUT analysis

CCD results suggested a linear model for PUT; Equation (2) predicted the responses for given levels of each variable in both boiling and frying methods.

where PUT is a concentration of putrescine; NaCl, amount of sodium chloride; Ni, amount of sodium nitrite; pp, amount of sodium polyphosphate and AA, amount of ascorbic acid.

Source	Sum of squares	d.f.	Mean square	F value	P value	
Model	1532.98	7	219.00	4739.27	< 0.0001	Significant
A-Sodium chloride	272.70	1	272.70	5901.52	< 0.0001	
B-Nitrite	1165.87	1	1165.87	25230.35	< 0.0001	
C-PP	84.58	1	84.58	1830.41	< 0.0001	
D-AA	7.91	1	7.91	171.10	< 0.0001	
E-Cooking	0.000	1	0.000	0.000	1.0000	
AC	1.41	1	1.41	30.44	< 0.0001	
BC	0.51	1	0.51	11.10	0.0016	
Residual	2.40	52	0.046			
Lack of fit	2.40	42	0.057			Not significant
Pure error	0.000	10	0.000			
Core total	1535.39	59	219.00			
R <sup>2</sup> = 0.9984						
Adjusted R <sup>2</sup> = 0.9982						
Adequate precision = 277.959						

Table 2. Analysis of variance for SPM content.



Figure 1. Perturbation graph showing linear effects of curing agents. A: NaCl, B: sodium nitrite, C: sodium polyphosphate, D: ascorbic acid.

Table 3 shows the ANOVA for PUT. A significant relationship between the curing process and PUT content (P < 0.0001) was noted. ANOVA analysis indicated that this model (linear) was also significant for PUT (F = 2491.24). Furthermore, lack of fit is not significant indicating no significant relation with a pure error. The coefficient determination obtained from ANOVA, exhibited a strong relationship between the raw data and the proposed model ( $R^2 = 0.9957$ ), and provided an index of how well-observed results were replicated by the model. This statistical tool also estimates variation in the data calculated by the model, 99.57% variation in the responses was explained by the proposed model, and the linear model

was compatible with the experimental data. Adjusted R<sup>2</sup> of 0.9953 represented the potential of the model to predict response. Adequate precision was 199.13, indicating adequate signal. All independent variables except E had a significant linear influence on PUT content (Table 3; P < 0.0001).

Sodium chloride (NaCl) is generally accepted as a good additive in the formulation of several food products. This study results indicated that the presence of salt in the cured-meat samples enhanced the reduction of PAs content. This outcome agreed with the study of Roseiro et al. (2017b) who observed a decrease in biogenic amine concentrations after the addition of NaCl to dry-cured tuna. Reduction of biogenic amines and PAs post-NaCl addition was also demonstrated in response to its antibacterial properties (Naila et al., 2010). NaCl is an efficient additive because it inhibits the growth of microorganisms, enhances the flavor, and has impacts on enzymatic, water activity, and the texture of animal tissues (Roseiro et al., 2017a). Although the recent policies of regulatory organizations like the WHO recommend decreasing NaCl level in food formulations because of its relationship with cardiovascular disorders and blood hypertension, it is better to optimize its level than completely removing it (Erdogan et al., 2018). Laranjo et al. (2017) found increased content of biogenic amines when the NaCl addition was reduced by 50% in dry-cured sausage formulation, which was directly influenced by the release of decarboxylase enzymes from bacteria.

Sodium nitrite is another additive playing an important role as a curing agent. It speeds up meat curing and preserves the cured meat from being spoiled by bacteria like

Table 3. Analysis of variance for PUT contents.

Source	Sum of squares	d.f.	Mean square	F value	P value	
Model	2317.19	5	463.44	2491.24	< 0.0001	Significant
A-Sodium chloride	544.12	1	544.12	2924.96	< 0.0001	0
B-Nitrite	1638.07	1	1638.07	8805.54	< 0.0001	
C-PP	128.03	1	128.03	688.24	< 0.0001	
D-AA	6.82	1	6.82	36.65	< 0.0001	
E-Cooking	0.1500	1	0.1500	0.8063	0.3732	
Residual	10.05	54	0.1860			
Lack of fit	4.84	44	0.1099	0.2111	0.9999	Not significant
Pure error	5.21	10	0.5208			
Core total	2327.23	59				
R <sup>2</sup> = 0.9957						
Adjusted R <sup>2</sup> = 0.9953						
Adequate precision = 199.13						

Clostridium botulinum which causes botulism (Alizadeh et al., 2020). Reducing effects of sodium nitrite on PAs were observed in this study. Our results matched the outcomes of Gençcelep et al. (2007) who demonstrated that nitrite inhibits the growth of amine-producing bacteria and hence reduced amines. Jastrzębska et al. (2016) added several additives into fresh white and red meat to evaluate their effects on some biogenic amines like PUT, SPD, SPM, and cadaverine. They reported that sodium nitrite and sodium chloride caused a reduction of biogenic amines formation by inhibiting amine-forming bacterial activities. Moreover, the inhibitory effect of sodium nitrite was determined stronger than sodium chloride, especially in poultry meat. It should be stressed that a steep slope in the perturbation graph and a greater coefficient of B term in the equation in this study outcomes are similar to the one reported in Jastrzębska et al.'s (2016) findings. Besides, Paulsen and Bauer (2007) studied the effects of nitrite and sodium chloride on SPD and SPM contents in pork meat and observed a significant reduction of PAs after the addition of nitrite that confirms our findings.

Another important curing agent is sodium polyphosphate. Its beneficial properties include prolongation of shelf life, this aids in retaining the color and flavor, increasing water holding capacity, halting lipid oxidation and microbial load, and improves texture stability, especially in combination with other curing additives like NaCl (Kilic *et al.*, 2018). Though data about the effects of polyphosphate on meat quality are widespread, studies on its effects on biogenic amines and PAs are rare. Bozkurt and Erkmen (2002) reported a decline in biogenic amines after adding phosphates with some other additives like nitrite, nitrate, a-tocopherol, ascorbic acid, and potassium sorbate to sausage, which was similar to the current study findings. Ascorbic acid is another common additive using in cured meat production as it has antioxidant effects and can also influence the color of the final product (Perlo *et al.*, 2018). But in this study, its influence in the reduction of PUT and SPM contents was minimal.

Thermal treatments were the last tested factor. They were recognized as diminishing agents too and led to a decrease in PAs content. Furthermore, a previous study revealed that higher temperatures resulted in a further reduction in PAs (Dadáková *et al.*, 2011). However, contrasting results were observed in this study, finding no significant changes in applying culinary treatments. It is presumed that heating effects are overlayed with other strong agents like sodium nitrite.

Significant interactive effects were also observed for SPM content described by the 2FI model. It indicated that each parameter might influence other factors. For example, the three-dimensional surface of the interactive effects of sodium chloride and sodium polyphosphate is depicted in Figure 2. It shows that when the amount of polyphosphate and sodium chloride increases, significant decrease in polyamine content is seen (P < 0.0001). It should be noted that cooking was at average mode, and the sodium nitrite and ascorbic acid concentrations were constant at their central points (100 and 250 ppm, respectively). The interaction effects of sodium nitrite and sodium polyphosphate on SPM showed a dramatic reduction in SPM content (Figure 3; P < 0.0001) when NaCl and ascorbic acid were steady in their central points (1 g and 250 ppm), and an average mode of cooking was used. Hence, polyamine content experienced a sharp decline after an increased addition of sodium nitrite and polyphosphate to samples (synergistic effect).



Figure 2. Three-dimensional graph of interactive effects of salt and sodium polyphosphate on SPM obtained from CCD indicating reduction when sodium nitrite (100 ppm) and ascorbic acid (250 ppm) are constant at their central points.



Figure 3. Three-dimensional graph of interactive effects of sodium nitrite and sodium polyphosphate on SPM obtained from CCD indicating reduction when sodium chloride (1 g) and ascorbic acid (250 ppm) are constant at their central points.



Figure 4. Ramp view for optimization curing agents' levels with propagation of error.

# Optimization of curing agents' concentrations

The main purpose of this study was the optimization of the concentration of curing agents by RSM based on CCD to minimize PAs level in breast turkey meat. Accordingly, independent variables were set in the range of studied level, and the responses were considered in the minimum. Moreover, propagation of error (POE) was chosen to make the optimum condition robust to variations in input variables and is only used for nonlinear models. Derringer's desirability function is another value applied for the selection of optimum curing conditions. The desirability function is a value implying the validity of the proposed condition and is acceptable above 0.7 (Afshari *et al.*, 2015; Granato and Ares, 2014). The optimum amount of sodium chloride, sodium nitrite, sodium polyphosphate, and ascorbic acid to minimize SPM and PUT content, was 2 g, 200 ppm, 0.5 g, and 382 ppm, respectively, in frying mode. This value for our proposed condition was equal to 0.963, indicating that the chosen solution was a great option. Figure 4 displays the ramp view for optimization levels with POE. Selected POE helped have minimum variation in factor setting to have more exact responses.



Figure 5. Chromatogram of cured turkey breast meat sample after cooking determined by HPLC-ultra-violet detector under optimal condition (1, PUT, 2, SPM).

Applying the optimal condition to the samples besides optimized DLLME proposed in our previous research showed having sharp, precise, with no overlap peaks chromatograms for PAs (Figure 5).

# Conclusion

The turkey breast meat was studied to investigate PA content for the first time. Applying industrial practice (meat curing) and thermal treatments, and then analyzing the data by RSM to evaluate changes after this kind of treatment was the aim of the study. In conclusion, sodium chloride, sodium nitrite, and sodium polyphosphate are strong agents decreasing PAs levels. Moreover, boiling and frying were not as powerful as the curing process in PAs reduction. The responses were fitted with 2FI and linear models where high R<sup>2</sup> and adequate precision were observed. This strongly indicated the validity of our models, so that the condition was optimized according the models. The proposed optimal condition to reduce PUT and SPM to the lowest level was frying in combination with 2 g NaCl, 200 ppm sodium nitrite, 0.5 g sodium polyphosphate, and 382 ppm ascorbic acid. Future research should concentrate on the evaluation of polyamine changes in terms of novel food processing like irradiation, cold plasma and so on in meat and other foods.

#### References

Afshari, R., Hosseini, H., Khaksar, R., Mohammadifar, M.A., Amiri, Z., Komeili, R., *et al.* 2015. Investigation of the effects of inulin and β-glucan on the physical and sensory properties of low-fat beef burgers containing vegetable oils: optimisation of the formulation using D-optimal mixture design. Food Technology and Biotechnology 53: 436–445. https://doi.org/10.17113/ ftb.53.04.15.3980

- Alizadeh, A.M., Hashempour-Baltork, F., Alizadeh-Sani, M., Maleki, M., Azizi-Lalabad, M., and Khosravi-Darani K. 2020. Inhibition of Clostridium (C.) botulinum and its toxins by probiotic bacteria and their metabolites: an update review. Quality Assurance and Safety of Crops & Foods 12: 59–68. https://doi. org/10.15586/qas.v12iSP1.823
- Amirkhanov, K., Igenbayev, A., Nurgazezova, A., Okuskhanova, E., Kassymov, S., Muslimova N., et al. 2017. Research article comparative analysis of red and white Turkey meat quality. Pakistan Journal of Nutrition 16: 412–416. https://doi.org/10.3923/pjn.2017.412.416
- Araújo, J. and de Aquino Santana, L., 2018. Predictive modelling of foodborne bacteria inhibition by pomegranate (Punica granatum L.) peel extracts using response surface methodology. Quality Assurance and Safety of Crops & Foods 10: 137–144. https://doi.org/10.3920/QAS2017.1187
- Bakhtiary, F., Sayevand, H.R., Remely, M., Hippe, B., Hosseini, H., Haslberger, A.G., et al. 2016. Evaluation of bacterial contamination sources in meat production line. Journal of Food Quality 39: 750–756. https://doi.org/10.1111/jfq.12243
- Bashiry, M., Mohammadi, A., Hosseini, H., Kamankesh, M., Aeenehvand, S., and Mohammadi, Z. 2016. Application and optimization of microwave-assisted extraction and dispersive liquid–liquid microextraction followed by high-performance liquid chromatography for sensitive determination of polyamines in turkey breast meat samples. Food Chemistry 190: 1168– 1173. https://doi.org/10.1016/j.foodchem.2015.06.079
- Bozkurt, H. and Erkmen, O., 2002. Effects of starter cultures and additives on the quality of Turkish style sausage (sucuk). Meat Science 61: 149–156. https://doi.org/10.1016/S0309-1740(01)00176-0
- Busboom, J.R., 2003. Curing and smoking poultry meat. Cooperative Extension, Washington State University.
- Dadáková, E., Pelikanova, T. and Kalac, P., 2011. Concentration of biologically active polyamines in meat and liver of sheep and lambs after slaughter and their changes in mutton during storage and cooking. Meat Science 87: 119–124. https://doi. org/10.1016/j.meatsci.2010.09.009
- Dadáková, E., Pelikánová, T. and Kalač, P., 2012. Concentration of biologically active polyamines in rabbit meat, liver and kidney after slaughter and their changes during meat storage and cooking. Meat Science 90: 796–800. https://doi.org/10.1016/j. meatsci.2011.11.017
- Dandrifosse, G., 2009. Biological aspects of biogenic amines, polyamines and conjugates. Transworld Research Network.
- El Adab, S., Wadda, W.B., Tekiki, A., Moussa, O.B., Boulares, M., et al. 2020. Effect of mixed startecultures on biogenic amines formation durin the ripening of Tunisian fermented camle meat sausage. Italian Journal of Food Science 321–336. https://doi. org/10.14674/IJFS-1733
- Erdogan, M., Agirman, B., Boyaci-Gunduz, C. and Erten, H., 2018. Partial replacement of sodium chloride with other chloride salts for the production of black table olives from cv. Gemlik. Quality Assurance and Safety of Crops & Foods 10: 399–410. https://doi. org/10.3920/QAS2018.1314
- Gençcelep, H., Kaban, G. and Kaya, M., 2007. Effects of starter cultures and nitrite levels on formation of biogenic amines in sucuk. Meat Science 77: 424–430. https://doi.org/10.3920/QAS2018.1314

- Granato, D. and Ares, G., 2014. Mathematical and statistical methods in food science and technology. John Wiley & Sons. https:// doi.org/10.1002/9781118434635
- Haddad, G.d.B.S., Moura, A.P.R., Fontes, P.R., Cunha, S.d.F.V.d., Ramos, A.d.L.S., *et al.* 2018. The effects of sodium chloride and PSE meat on restructured cured-smoked pork loin quality: a response surface methodology study. Meat Science 137: 191– 200. https://doi.org/10.1016/j.meatsci.2017.11.030
- Handa, A.K., Fatima, T. and Mattoo, A.K., 2018. Polyamines: bio-molecules with diverse functions in plant and human health and disease. Frontiers in Chemistry 6;10, 1–18. https://doi. org/10.3389/fchem.2018.00010
- Hazar, F.Y., Kaban, G. and Kaya, M., 2017. The effects of different processing conditions on biogenic amine formation and some qualitative properties in pastirma. Journal of Food Science and Technology 54: 3892–3898. https://doi.org/10.1007/ s13197-017-2845-8
- Hrynets, Y., Omana, D.A., Xu, Y. and Betti, M., 2011. Impact of citric acid and calcium ions on acid solubilization of mechanically separated turkey meat: effect on lipid and pigment content. Poultry Science 90: 458–466. https://doi.org/10.3382/ ps.2010-00859
- Jastrzębska, A., Kowalska, S. and Szłyk, E., 2016. Studies of levels of biogenic amines in meat samples in relation to the content of additives. Food Additives & Contaminants: Part A 33: 27–40. https://doi.org/10.1080/19440049.2015.1111525
- Kalač, P., 2009. Recent advances in the research on biological roles of dietary polyamines in man. Journal of Applied Biomedicine 7: 65–74. https://doi.org/10.32725/jab.2009.007
- Kalač, P., 2014a,b. Health effects and occurrence of dietary polyamines: a review for the period 2005–mid 2013. Food Chemistry 161: 27–39. https://doi.org/10.1016/j.foodchem.2014.03.102
- Kamani, M.H., Safari, O., Mortazavi, S.A. and Atash, M.M.S., 2015. Predicting the contents of volatile and non-volatile amines in rainbow trout fillet during storage time via image processing technique. Quality Assurance and Safety of Crops & Foods 7: 589–598. https://doi.org/10.3920/QAS2014.0445
- Keşkekoğlu, H. and Üren, A., 2013. Formation of biogenic amines during fermentation and storage of tarhana: a traditional cereal food. Quality Assurance and Safety of Crops & Foods 5: 169– 176. https://doi.org/10.3920/QAS2012.0150
- Kilic, B., Simsek, A., Claus, J.R., Karaca, E. and Bilecen, D., 2018. Improving lipid oxidation inhibition in cooked beef hamburger patties during refrigerated storage with encapsulated polyphosphate incorporation. LWT 92: 290–296. https://doi. org/10.1016/j.lwt.2018.02.037
- Kozová, M., Kalač, P. and Pelikánová, T., 2009. Contents of biologically active polyamines in chicken meat, liver, heart and skin after slaughter and their changes during meat storage and cooking. Food Chemistry 116: 419–425. https://doi.org/10.1016/j. foodchem.2009.02.057
- Laranjo, M., Gomes, A., Agulheiro-Santos, A.C., Potes, M.E., Cabrita, M.J., et al. 2017. Impact of salt reduction on biogenic amines, fatty acids, microbiota, texture and sensory profile in traditional blood dry-cured sausages. Food Chemistry 218: 129– 136. https://doi.org/10.1016/j.foodchem.2016.09.056

- Mejri, J., Melki, M. and Mejri, M., 2017. Modeling of cooking and coolind processes of Tunisian salami. Italian Journal of Food Science 29: 613–626. https://doi.org/10.14674/IJFS-665
- Mokhtarian, M., Heydari Majd, M., Koushki, F., Bakhshabadi, H., Daraei Garmakhany, A., *et al.* 2014. Optimisation of pumpkin mass transfer kinetic during osmotic dehydration using artificial neural network and response surface methodology modelling. Quality Assurance and Safety of Crops & Foods 6: 201–214. https://doi.org/10.3920/QAS2012.0121
- Muñoz-Esparza, N.C., Latorre-Moratalla, M.L., Comas-Basté, O., Toro-Funes, N., Veciana-Nogués, M.T., *et al.* 2019. Polyamines in food. Frontiers in Nutrition 6: 108–108. https://doi.org/10.3389/ fnut.2019.00108
- Naila, A., Flint, S., Fletcher, G., Bremer, P. and Meerdink, G., 2010. Control of biogenic amines in food—existing and emerging approaches. Journal of Food Science 75: R139–R150. https://doi. org/10.1111/j.1750-3841.2010.01774.xc
- Naseri, M., Rezaei, M., Moieni, S., Hosseni, H. and Eskandari, S., 2010. Effect of different precooking methods on chemical composition and lipid damage of silver carp (Hypophthalmichthys molitrix) muscle. International Journal of Food Science & Technology 45: 1973–1979. https://doi.org/10.1111/j.1365-2621.2010.02349.x
- Pandiselvam, R., Manikantan, M.R., Sunoj, S., Sreejith, S. and Beegum, S., 2019. Modeling of coconut milk residue incorporated rice-corn extrudates properties using multiple linear regression and artificial neural network. Journal of Food Process Engineering 42: e12981. https://doi.org/10.1111/jfpe.12981
- Paulsen, P. and Bauer, F., 2007. Spermine and spermidine concentrations in pork loin as affected by storage, curing and thermal processing. European Food Research and Technology 225: 921–924. https://doi.org/10.1007/s00217-006-0464-0
- Perlo, F., Fabre, R., Bonato, P., Jenko, C., Tisocco, O., *et al.* 2018. Refrigerated storage of pork meat sprayed with rosemary extract and ascorbic acid. Ciência Rural 48:04;1–7. https://doi. org/10.1590/0103-8478cr20170238
- Pilevar, Z., Bahrami, A., Beikzadeh, S., Hosseini, H. and Jafari, S.M., 2019. Migration of styrene monomer from polystyrene packaging materials into foods: characterization and safety evaluation. Trends in Food Science & Technology 91: 248–261. https://doi. org/10.1016/j.tifs.2019.07.020
- Posthuma, J.A., Rasmussen, F.D. and Sullivan, G.A., 2018. Using a cured meat model system to investigate factors that influence cured color development. Nebraska Beef Cattle Report; 128–131.
- Ramezani, H., Hosseini, H., Kamankesh, M., Ghasemzadeh-Mohammadi, V. and Mohammadi, A., 2015. Rapid determination of nitrosamines in sausage and salami using microwave-assisted extraction and dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry. European Food Research and Technology 240: 441– 450. https://doi.org/10.1007/s00217-014-2343-4
- Roseiro, L.C., Santos, C., Goncalves, H., Serrano, C., Aleixo, C., et al. 2017a. Susceptibility of dry-cured tuna to oxidative deterioration and biogenic amines generation: I. Effect of NaCl content, antioxidant type and ageing. Food Chemistry 228: 26–34. https://doi.org/10.1016/j.foodchem.2017.01.125

- Roseiro, L.C., Santos, C., Gonçalves, H., Serrano, C., Aleixo, C., et al. 2017b. Susceptibility of dry-cured tuna to oxidative deterioration and biogenic amines generation: I. Effect of NaCl content, antioxidant type and ageing. Food Chemistry 228: 26–34. https://doi.org/10.1016/j.foodchem.2017.01.125
- Sagarika, N., Prince, M.V., Kothakota, A., Pandiselvam, R., Sreeja, R., et al. 2018. Characterization and optimization of microwave assisted process for extraction of nutmeg (Myristica fragrans Houtt.) mace essential oil. Journal of Essential Oil Bearing Plants 21: 895–904. https://doi.org/10.1080/0972060X.2018.1517613
- Sebranek, J.G. and Bacus, J.N., 2007. Cured meat products without direct addition of nitrate or nitrite: what are the issues? Meat Science 77: 136–147. https://doi.org/10.1016/j.meatsci.2007.03.025
- Shameena Beegum, P.P., Manikantan, M.R., Sharma, M., Pandiselvam, R., Gupta, R.K., *et al.* 2019. Optimization of processing variables for the development of virgin coconut oil cake based extruded snacks. Journal of Food Process Engineering 42: e13048. https://doi.org/10.1111/jfpe.13048
- Srikanth, V., Rajesh, G.K., Kothakota, A., Pandiselvam, R., Sagarika, N., *et al.* 2020. Modeling and optimization of developed cocoa beans extractor parameters using box behnken design and artificial neural network. Computers and Electronics in Agriculture 177: 105715. https://doi.org/10.1016/j.compag. 2020.105715
- Srinivas, Y., Mathew, S.M., Kothakota, A., Sagarika, N. and Pandiselvam, R., 2020. Microwave assisted fluidized bed drying of nutmeg mace for essential oil enriched extracts: an assessment of drying kinetics, process optimization and quality.

Innovative Food Science & Emerging Technologies 66: 102541. https://doi.org/10.1016/j.ifset.2020.102541

- Taheri, T., Fazlara, A., Roomiani, L. and Taheri, S., 2018. Effect of chitosan coating enriched with cumin (Cuminum cyminum L.) essential oil on the quality of refrigerated turkey breast meat. Italian Journal of Food Science 30: 628–640. https://doi.org/10.14674/IJFS-1158
- Triki, M., Herrero, A.M., Jiménez-Colmenero, F. and Ruiz-Capillas, C., 2018. Quality assessment of fresh meat from several species based on free amino acid and biogenic amine contents during chilled storage. Foods 7: 132. https://doi.org/10.3390/ foods7090132
- Vidal, V.A.S., Biachi, J.P., Paglarini, C.S., Pinton, M.B., Campagnol, P.C.B., *et al.* 2019. Reducing 50% sodium chloride in healthier jerked beef: an efficient design to ensure suitable stability, technological and sensory properties. Meat Science 152: 49–57. https://doi.org/10.1016/j.meatsci.2019.02.005
- Xu, N., Li, T., Jia, R., Zhang, H. and Wang, R., 2018. Selection of nitrite and bioamine-degrading bacteria and its improvement of fish sausage quality. Nongye Gongcheng Xuebao/Transactions of the Chinese Society of Agricultural Engineering 34: 304–312. https://doi.org/10.11975/j.issn.1002-6819.2018.15.038
- Zhang, Y.M., Qin, N., Luo, Y.K. and Shen, H.X., 2015. Effects of different concentrations of salt and sugar on biogenic amines and quality changes of carp (Cyprinus carpio) during chilled storage. Journal of the Science of Food and Agriculture 95: 1157–1162. https://doi.org/10.1002/jsfa.6803

Runs	Sodium chloride (gram)	Sodium nitrite (ppm)	Sodium polyphosphate (gram)	Ascorbic acid (ppm)	Cooking
1	1	100	0.25	250	Boiling
2	0	0	0	500	Frying
3	1	100	0.25	250	Boiling
4	2	200	0.5	500	Boiling
5	1	100	0.25	250	Frying
6	1	100	0.25	125	Frving
7	0.5	100	0.25	250	Frving
8	0	0	0.5	500	Boiling
9	0	0	0	500	Boiling
10	2	200	0	0	Boiling
11	0	0	0.5	0	Frvina
12	1	100	0.25	250	Frvina
13	2	0	0	0	Frvina
14	2	0	0	500	Boiling
15	0	200	0	500	Boiling
16	1	100	0.375	250	Frving
17	15	100	0.25	250	Frving
18	1.0	50	0.25	250	Frving
10	1	100	0.25	125	Boiling
20	2	200	0.5	500	Erving
20	2	200	0	500	Boiling
21	0	200	0.5	0	Erving
22	1	150	0.25	250	Boiling
23	0	200	0.23	0	Boiling
24	1	200	0.25	275	Enving
20	1	100	0.25	250	Frying
20	0	100	0.25	250	Poiling
21	1	100	0.25	250	Enving
20	0	200	0.25	200	Frying
29	1	200	0.25	250	Poiling
21	1	100	0.25	250	Dolling
20	1	200	0.25	200	Dulling
3Z 22	2	200	0	500	Frying
33 24	2	200	0.5	0	Boiling
34 25		100	0.25	250	Frying
30	2	100	0.5	500	Boiling
30 27	1	100	0.125	250	Boiling
31 20	1	50	0.25	250	Boiling
30	1	100	0.25	250	Boiling
39	0	200	0.5	500	Boiling
40	0	200	0	0	Frying
41	1	100	0.125	250	Frying
42	2	0	0	500	Frying
43		100	0.25	3/5	Boiling
44	2	200	0	0	Frying
45	0.5	100	0.25	250	Bolling
40	2	0	0	0	Bolling
47	0	200	0.5	500	Frying
48	2	0	0.5	500	Frying

Appendix A. CCD to investigate curing agents and heat process effects on polyamine contents.

Bashiry	М	et	al.
---------	---	----	-----

Runs	Sodium chloride (gram)	Sodium nitrite (ppm)	Sodium polyphosphate (gram)	Ascorbic acid (ppm)	Cooking
49	0	0	0	0	Frying
50	1.5	100	0.25	250	Boiling
51	1	100	0.25	250	Boiling
52	2	0	0.5	0	Boiling
53	0	0	0.5	500	Frying
54	1	150	0.25	250	Frying
55	2	0	0.5	0	Frying
56	1	100	0.375	250	Boiling
57	1	100	0.25	250	Frying
58	0	200	0.5	0	Boiling
59	2	200	0.5	0	Frying
60	0	0	0.5	0	Boiling