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Nitrite reduction in sausage using tomato powder and *Satureja bachtiarica Bunge* essential oil by response surface methodology

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ABSTRACT

Background & Aim: The use of synthetic additives is one of the main approaches for preventing microbial growth and oxidative reactions in meat products. These preservatives were recently marked as unhealthy to humans; therefore, the consumers demand for fresh, natural, and negligibly processed products with lower content of artificial additives is increasing.
Experimental: The effect of *Satureja bachtiarica Bunge* essential oil (EO) and tomato powder (TP) to optimize sausage formulation with reduced nitrite content was investigated. Response surface methodology (RSM) was used to investigate the effects of different levels of EO (200-400 ppm), TP (5-15%) as Hurdles, and sodium nitrite (SN) (0-300 ppm) in sausage formulation during storage (0- 24 days). Dependent variables including residual nitrite, pH, color indices, microbial load, and hardness were investigated and finally model optimization and validation were conducted.
Results: The results showed that residual nitrite was strongly depending on initial added nitrite and storage time ($P<0.001$), so the use of nitrite substituent was undeniable to have an improved sausage formulation without microbial defect. RSM represented a quadratic model for all responses except in texture which was linear and the pH and microbial load showed interaction. The optimized predicted values for SN, EO and TP were 56 ppm, 378 ppm and 5%, respectively. The model validation revealed that the results of the experiments were in good agreement with the predicted values.
Recommended applications/industries: The results of the present study can be useful for consideration in meat products industry to reduce the nitrite level in sausage formulation.

1. Introduction

Hurdle technology is one of the new methods in food processing which uses several low-intensity agents instead of a high-intensity inhibitor to reduce the adverse effects on sample quality characteristics. One of the most important hurdles used in processed meats is nitrite and nitrate salts (Leistner, 2000, Qiu *et al.*, 2019). Sodium nitrite is a compound with antioxidant properties in addition to its antimicrobial properties. It also improves color and texture and enhances thermal stability in processed meats. Despite the positive effects of nitrite on the quality and microbial properties of the end product, residual nitrite causes a possible formation of carcinogens (N-nitrosamine) (Honikel, 2008; Hospital *et al.*, 2014).

Thus, numerous studies on nitrite reduction in meat products by the use of nitrite substitutes were successfully conducted in recent years (Alirezalu *et al.*, 2018; Aquilani *et al.*, 2018; Sucu and Turp, 2018; Kim *et al.*, 2019; Šojić *et al.*, 2019, Ghafouri-Oskuei *et al.*, 2020; Huang *et al.*, 2020).

Herbal extract compounds, various essential oils (oregano, savory, thyme, rosemary), and herbal powder such as tomato and blueberry powder, organic acids such as acetate, lactate, sorbate, and benzoate, bacteriocins as well as high pressure process are all alternatives to nitrite (Amali *et al.*, 2015)

Herbal extracts and essential oils were widly used in cured meat because of antimicrobial and anti oxidant properties of them (Nevas *et al.*, 2004; Oliveira *et al.*, 2012, Silveira *et al.*, 2014; Sojic *et al.*, 2015; Alirezalu *et al.*, 2018; Aquilani *et al.*, 2018; Araújo *et al.*, 2018; Ghafouri-Oskueii *et al.*, 2020; Huang *et al.*, 2020). For example Šojić *et al.* (2019) investigated the effect of Coriander essential oil on color, pH, lipid oxidation, residual nitrite, and microbial growth of sausages. They found that the effect of Coriander essential oil will ultimately reduce the amount of nitrite consumption while maintaining the quality properties of the product (Šojić *et al.*, 2019). Alirezalu *et al.* (2019) examined the effect of nisin, polylysine, and chitosan in combination with green tea, stinging nettle, and olive leaves extracts on the quality of nitrite-free frankfurters during freezing. They selected a combination of 0.2% polylysine and 1% chitosan with the mentioned extracts as the best nitrite replacement (Alirezalu *et al.*, 2018).

Other utilization of plants and fruits as nitrite alternatives are in the form of powder, pomace or fiber (Candogan, 2002; Garcia *et al.*, 2002; Deda *et al.*, 2007, Garcia *et al.*, 2007, Fernández-López *et al.*, 2008, Eyiler and Oztan, 2011; Kim *et al.*, 2011; Riazi *et al.*, 2016; Yadav *et al.*, 2016; Aquilani *et al.*, 2018; Sucu and Turp, 2018; Ahmad *et al.*, 2019; Ghafouri-Oskuei *et al.*, 2020). Tomato powder and tomato paste were used in some of these researches. Tomato powder as functional additive has been used in meat products for enhancing the existing characteristics and to develop a new product. The advantage of using tomato powder is that in addition to reusing tomato paste waste, it improves the color of the products and contains active ingredients such as phenolic compounds, organic acids, and carotenoids which can fulfill technical goals and improve the nutritional characteristics of the sausage (Eyiler and Oztan, 2011; Melendres *et al.*, 2014).

For example Ghafoori Oskooyi *et al.* (2019) investigated the effect of different levels of tomato powder and flaxseed on the sensory and chemical properties of meat sausages. They showed that the addition of tomato powder and flaxseed to 3% had no adverse effect on sensory characteristics of the sausage and reduced nitrite content during storage (Ghafouri-Oskuei *et al.*, 2020).

Satureja bachtiarica, an endemic species with relatively wide distribution, is traditionally used as a medicinal and spice plant in Iran. In earlier studies, essential oil of the plant, which contains biologically active components such as carvacrol and thymol, has shown a wide range of biological activities including antibacterial, antifungal and antioxidant which may have the great importance in several fields from food chemistry to pharmacology and pharmaceutics (Salehi-Arjmand *et al.*, 2014).

In the present study addition of these new ingredients (tomato powder and *Satureja bachtiarica* essential oil) in order to maintain the sensory quality and textural properties of sausage was optimized using response surface methodology to develop a safe product with reduced nitrite content as well as attractive color.

2. Materials and Methods

2.1. Sample preparation

In this study, sausage with 40% meat was prepared in Shaqayeq factory in Chahar Mahal and Bakhtiari province, Iran. In order to preparation of tomato powder, tomatoes were purchased from a local market. They were washed, sliced, and dried in an oven (Behdad, 3493, Iran) at 60℃ and then ground. Essential oil was extracted from the savory (*Satureja bachtiarica)* plant by Clevenger apparatus in Islamic Azad university laboratory, Chaharmahal and Bakhtiari province, Iran.

2.2. Experimental design

A 4-factor-3-level D-optimal experimental design was adopted for the optimization of levels of ingredients in which a total of 25 different trials were conducted in Table 1.

Table1. Levels of independent variables.

Run	SN (ppm)	TP (%)	EO (ppm)	Storage time (day)
1	300	5	307.4	18.9
2	0	5	400	24
3	0	15	280.03	24
4	174.63	9.18	290.73	6
5	300	5	307.4	18.9
6	125.68	15	375	12.81
7	0	5	311.22	0
8	180.14	15	200	12.79
9	300	12.12	200	24
10	300	15	311.4	0
11	300	5	400	0
12	0	15	280.03	24
13	300	5	200	0
14	152.86	9.9	372.12	24
15	300	15	400	24
16	0	8.99	200	12.81
17	0	5	400	24
18	295.31	10.91	400	9.75
19	0	15	400	0
20	0	9.58	346.83	12.92
21	300	15	311.4	0
22	300	5	400	0
23	85.02	5	200	24
24	103.71	5.78	200	0
25	0	15	200	0

SN: Sodium nitrite, TP: tomato powder, EO: essential oil.

The independent variables and their levels selected for the study were sodium nitrite (SN) content (0-300 ppm), tomato powder (TP) (5-15%), essential oil (EO) (200-400 ppm) and storage time (0-24 day).

The experimental plan was designed and the results obtained were analyzed using Design Expert version 7.1.6 (Stat-Ease Inc., Minneapolis, MN) software to build and evaluate models.

The 25 treatments provided by the software were tested as follows for assessing pH levels, residual nitrite content, microbial load, texture, and color indices L*, a *, b. All dependent variables were measured in at least two replicates and the average values were inserted in Table 2.

2.3. pH measurement

The pH of the sausage samples was determined using a pH-meter (Jenway, 3510) by direct measurement with a glass electrode calibrated with the phosphate buffers 4.0 and 7.0 at room temperature. A total of 10 g of homogenized sausage sample was thoroughly mixed in a 200 mL beaker with 90 g of distilled water, and the pH was measured with accuracy of 0.01%. The pH meter had a temperature correction system and no temperature adjustment was required, so the temperature was set by the device at 20±2 °C.

Table 2. Amount of dependent variables at different run.

run	pH	Residual nitrite	L	a^*	b^*	Hardness	Microbial load (cfu/g)
1	5.71	60	66.2	7.9	20.9	2687.6	0
2	5.36	0	59.6	8.4	24.3	2598.4	760000
3	5.18	0	63.5	7.8	22.5	2355.2	2400000
4	5.53	105	63.2	8.2	22.7	2541.5	100000
5	5.71	60	63.2	7.9	21.9	2687.6	0
6	5.22	36	62.2	9.1	21.6	2345.4	870000
7	5.74	0	65.8	7.7	22.3	2687.6	0
8	5.23	63	63.2	9.5	23.9	2344.9	490000
9	5.35	42	59.4	10.6	25.6	2445.7	650000
10	5.27	260	58.1	10.6	24.8	2334.5	0
11	5.75	240	62.3	7.9	22.9	2677.9	190000
12	5.18	0	63.5	7.8	22.6	2355.2	2000000
13	5.77	280	63.7	8.4	22.7	2679.4	210000
14	5.43	18	58.7	8.7	23.8	2525.3	650000
15	5.22	43	55.8	10.5	24.1	2345.3	0
16	5.51	0	60.6	9.4	24.6	2357.2	700000
17	5.43	0	59.6	9.4	24.3	2698.4	760000
18	5.44	159	54.2	9.4	24.1	2479.4	0
19	5.25	0	59.1	10.7	22.4	2342.8	610000
20	5.47	0	62.4	8.9	22.6	2535.9	860000
21	5.27	276	58.1	10.6	25.8	2334.5	0
22	5.65	275	62.3	7.9	22.9	2677.9	190000
23	5.67	5.19	60.5	8.8	24.5	2697.3	640000
24	5.71	103	62.9	8.1	23.7	2658.4	0
25	5.26	0	58.9	9.7	24.3	2344.3	130000

2.4. Measurement of residual nitrite content

The nitrite content in the prototype and the residual nitrite content (mg $NaNO_2$.kg sample) were measured using the standard method ISO 2918 :1975 (ISO 1975-09, ISO 2003a).

2.5. Microbial tests

The microbial analysis involved total plate count (TPC) (ISO 4833: 2003a, 2003b), and *Clostridium perfringens* count (ISO 7937: 2004).

2.6. Color Measurement of the samples

Color measurements were performed using a Hunterlab device (ColorFlex EZ, FMS Jansen GmbH) pre-calibrated by white and black tiles where 3 factors of L (lightness), a^*(redness), and b^*(yellowness) were determined.

2.7. Texture measurement

Texture analysis was performed at room temperature, using Brook Field CT3 4500 (USA) texture analyzer equipped with 50 kg load cell and a 5 cm flat cylindrical probe. Samples of sausages, which were 5 cm thick and 3 cm in diameter were compressed to 50% of their initial thickness at a constant test speed of 2 mm/s. Hardness was reported in terms of grams of force.

3. Results and discussion

Table 2 represents the results obtained from physicochemical analysis of 25 formulated sausage samples including pH, residual nitrite content, color indices of L, a^*, b^*, hardness and total count of microorganisms according to RSM runs. All statistical analyzes of data were performed at the significance level of P<0.05.

In order to optimize the sausage production conditions, the effect of independent variables including A: nitrite content (ppm), B: tomato powder (%), C: essential oil (ppm) and D: time (day) was examined. The RSM method defines a model for each dependent variable which states the main and interactive effects of the factors on each individual variable. The multivariate model is as follows.

Equation 1:

$$Y=\beta_0+\beta_a A+\beta_b B+\beta_c C+\beta_{aa}A^2+\beta_{bb}B^2+\beta_{cc}C^2+\beta_{ab}AB+\beta_{ac}AC+\beta_{bc}BC$$

Where, Y represents the predicted response, where β_0 is constant coefficient, β_a, β_b, β_c are linear coefficients, β_{aa}, β_{bb}, β_{cc} are quadratic coefficients, and β_{ab}, β_{ac}, β_{bc} are interaction coefficients.

After conducting the runs, the RSM proposes a model with low standard deviation (S.D.) and low predicted residual error sum of squares (PRESS) as well as a high correlation coefficient (R^2) for each response.

3.1. pH analysis

According to equation 2, the analysis of variance (ANOVA) indicated that the dual interaction factor model was statistically significant and fitted to represent the actual relationship between the pH in sausage and significant variables with a very small P value (<0.0001) and a satisfactory coefficient of R^2 (0.9764).

Equation 2:

$$pH= 5.45-0.028\ A- 0.22\ B -0.032\ C- 0.044\ D+ 0.026AD- 0.039\ BC$$

Table 3 showed that the linear effect of all process variables and also the interactions of SN-time and TP-EO were statistically significant on pH (P<0.05).

Table 3. Analysis of variance (ANOVA) and p value of the independent variables.

	pH	Residual nitrite	L	a*	b*	Microbial load	Hardness
A	0.0163	0.0001<	0.0126	0.0036	0.0091	0.0001<	0.3161
B	<0.0001	0.8132	<0.0001	<0.0001	0.0003	0.0001<	0.0001<
C	0.0124	0.1541	0.0043	0.6944	0.0001<	0.2857	0.5739
D	0.0007	<0.0001	0.2994	0.1784	0.7583	0.0001<	0.8836
AB	0.1583	0.2419	0.0177	0.0009	0.0001<	0.0001<	---
AC	0.1139	0.9967	0.0060	0.0828	0.0257	0.0045	---
AD	0.0385	<0.0001	0.2698	0.1433	0.0047	0.0001<	---
BC	0.0103	0.0569	0.0590	0.0998	0.0323	0.6203	---
BD	0.2080	0.6385	0.0003	0.0004	0.0007	0.0001<	---
CD	0.1552	0.6681	0.1926	0.2788	0.4845	0.001	---
A2	---	0.1250	0.0346	0.0080	0.0536	---	---
B2	---	0.0998	0.0006	0.1018	0.0003	---	---
C2	---	0.2095	0.0001<	0.0002	0.0001<	---	---
D2	---	0.0074	0.0080	0.3895	0.0002	---	---
model	0.0001<	0.0001<	0.0001<	0.0001<	0.0001<	0.0001<	0.0001<
R^2	0.9764	0.9966	0.9532	0.9604	0.9703	0.9769	0.9294

A: nitrite content, B: tomato powder, C: essential oil and D: time

While SN and the interactions of SN-time and TP-EO had positive effect on pH, TP, EO and time had a negative effect which is obvious in Figure 1(a, b).

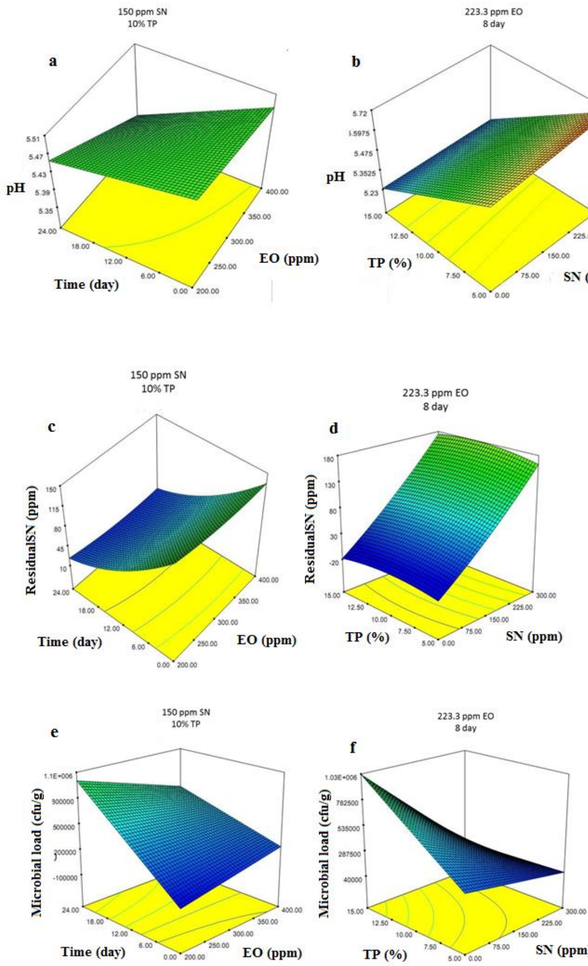

Figure 1. Three-dimensional response surface diagram for pH variations (a, b), residual nitrite (c, d) and microbial load changes (e, f).

The decrement of pH during storage is probably due to the growth of lactic acid bacteria and the fermentation of sugar to lactic acid and other organic acid. Sojic *et al.* (2019) and Deda *et al.* (2007) reported the same results on reducing pH during storage time of frankfurters (Deda *et al.*, 2007; Šojić *et al.*, 2019). The positive relation of SN and pH during the storage time was due to positive relation of added SN in the formulation and residual nitrite in the product during storage. Higher amount of nitrite in the product inhibited the growth of lactic acid bacteria so pH reduction would be restricted.

An important alternative to nitrite could be the use of tomato plant products that reduce the pH of sample because of its acidic nature. Many similar studies have been done on adding tomato powder and other tomato products as an alternative to sodium nitrite and all of them reported the reduction of pH by adding such component (Candogan, 2002; Deda *et al.*, 2007; Eyiler and Oztan, 2011; Kim *et al.*, 2011; Yadav *et al.*, 2016; Ghafouri-Oskuei *et al.*, 2020).

3.2. Residual nitrite in sausage

According to equation 3, the analysis of variance (ANOVA) indicated that the quadratic model was statistically significant and fitted to represent the actual relationship between the residual nitrite in sausage and significant variables with a very small P value (<0.001) and a satisfactory coefficient of R^2 (0.9966).

Equation 3:

$$\text{Residual nitrite} = 56.6 + 72.7 - 58.3\,D - 60.6\,AD + 17.39\,D^2$$

Only the linear effect of SN content, quadratic effect of storage time and SN-time interaction, were significant (P<0.01). The response surface diagram (Figure 1 c, d) indicated that the residual nitrite content increased by addition of SN content while the SN content decreased over time. The formation rate of nitrosamines was directly related to the squared concentration of SN in the product. Thus, the formation of nitrosamines can be significantly reduced by lowering even a small amount of added SN. The residual SN content during the process and during storage was reduced due to its combination with meat myoglobin and the production of nitroso myochromogen and other factors such as the effect of microbial load, enzyme, oxidation to nitrate etc (Honikel, 2008; Amali *et al.*, 2015).

3.3. Color factors

As color of a product is an important and most probably the first attribute influencing consumer acceptance, it was mentioned in our study. Equation 4 showed that the quadratic model was fitted by RSM to relate L factor and significant variables with a very small P value (<0.0001) and acceptable R^2 (0.9532).

Equation 4:

$$L = 63.1 - 0.7A - 1.5B - 0.9C - 0.8AB - 1.1AC + 1.5BD - 1.3A^2 + 2.8B^2 - 2.8C^2 - 1.8D^2$$

Table 3 indicated that the effects of SN, TP, EO (linear), all quadratic effects and the interactions of SN-TP, SN-EO and TP-time were statistically significant ($P<0.05$). Almost all significant variables had a negative effect on L factor. Analysis of variance indicated that there was a strong relation between the a^* factor and the variable response ($P<0.0001$ and R^2= 0.9604) and the quadratic model was fitted by RSM according to equation 5.

Equation 5:

$$a^*=8.21+0.29A+0.74B+0.43AB-0.47BD+0.59A^2+0.84C^2$$

Table 3 represented that linear positive effects of SN and TP, quadratic effects of SN and EO and the interactions of SN-TP and TP-time were statistically significant ($P<0.01$) on a^* factor. The significant ($P<0.05$) independent variables on b^* factor according to Table 3 were linear effects of SN, TP and EO, all interactions except EO-time and all quadratic effects except SN.

Equation 6:

$$b^*=22.5+0.3A+0.4B-0.6C+0.9AB+0.3AC-0.3AD-0.3BC-0.4BD-1.1B^2+0.9C^2+1.1D^2$$

The addition of non-meat ingredients to meat products can affect the color of the product (King, 2008). L factor increased by increasing SN and decreasing TP while a^* and b^* increased by increasing in both SN and TP content. Increasing in storage time increased L and b^* but had not any effect on a^* (Figure 2). The reduction of L factor by increasing TP is probably due to dark nature of the powder used. The redness of sausage sample was strongly related to TP content ($P<0.0001$) because of high amount of red pigment (lycopene) in TP. Also, SN extremely affected the red color which was due the higher amount of the red nitrosylmyoglobin formed in the presence of nitric oxide (NO) reduced from sodium nitrite and myoglobin (Wirth, 1986). Finally, the highest amount of a^* factor was at the highest amount of SN and TP. Similar results have been reported by Deda *et al.* (2007) for frankfurters produced with different levels of tomato paste (0-16%) and two levels of sodium nitrite (0 and 150 ppm) (Deda *et al.*, 2007). Similar results are reported on the effects of the addition of tomato products and sodium nitrite on color of meat products in other researches (Candogan, 2002; Eyiler and Oztan, 2011; Ghafouri-Oskuei *et al.*, 2020).

The EO had an inverse significant ($P<0.01$) effect on L, and b* indices due to the dark and green nature of *saturja bunga* EO. The highest L-value achieved in the middle of storage and at EO around 300 ppm at highest SN and lowest TP content (Figure 2 a, b).

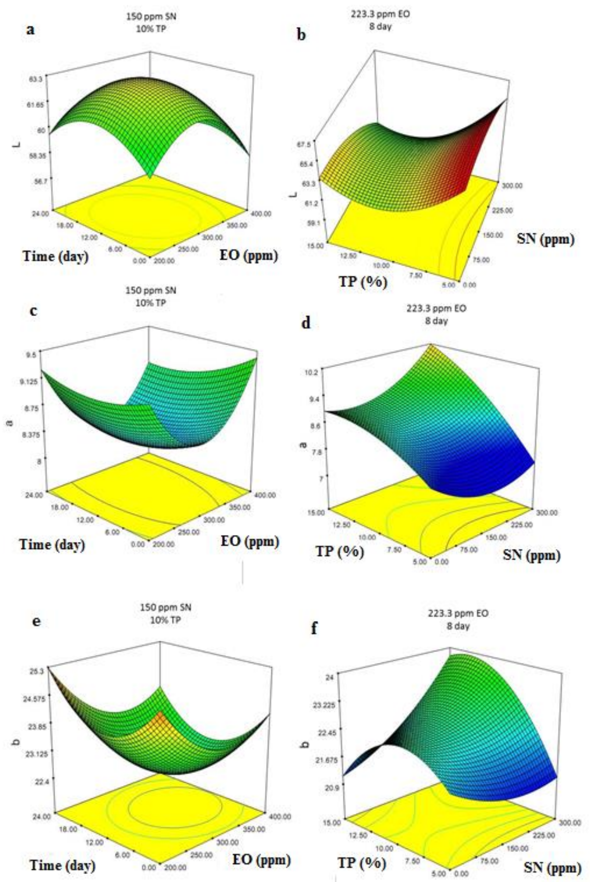

Figure 2. Three-dimensional response surface diagram for color attitudes: L (a, b), a* (c, d) and b* (e, f).

Oliviera *et al.* (2012) indicated that the effect of *Satureja montana* L. essential oil on color indices of martadella sausage was affected by sodium nitrite content and the concentration of EO. Lightness was lower at 100 mg/kg sodium nitrite rather than 200 mg/kg. The concentration more than 15.60 showed adverse effect on the color and reduced a* and increased b* indices. The color fading at higher amount of EO was probably due to the interaction of nitrite and aromatic component of EO that made NO_2- unavailable to combine with myoglobin. Another reason was the pro-oxidant effect of higher amount of EO and separating nitric oxide from nitrosylmyoglobin so producing brown myoglobin (Oliveira *et al.*, 2012).

3.4. Microbial evaluation of sausages

3.4.1. Clostridium perfringens count

The results of identification and count of *Clostridium perfringens* showed the lack of this microorganism in all treatments and days of storage, indicating that sausage production was in good heating condition and showed a good quality.

3.4.2. Total microbial count

In examining the changes in microbial count the analysis of variance (ANOVA) indicated that the dual interaction factor model (equation 7) was statistically significant and fitted to represent the actual relationship between the total microbial load and significant responses ($P<0.0001$ and $R^2 =0.9769$) .

Equation 7:

Microbial load= 100×(4664 - 3557 A+2426 B+ 3468 D – 1961 AB – 1303 AC – 3190 AD + 2047 BC – 1557 CD)

According to Table 3, it was found that the linear effects of SN, TP contents and time and the interactions of SN-TP, SN-EO, SN-time, TP-time, EO-time were significant ($P<0.05$) on microbial load of the sausage. According to the response surface diagram (Figure 1 e, f), antimicrobial effect of EO was revealed during the storage time. At the beginning of the storage, EO did not affect microbial load but at the last day of storage microbial load decreased by increasing in EO. At the other hand at low percentage of TP (5%), different amount of SN content had not sensible effect on microbial load that indicated the effect of TP on inhibiting the growth of microorganism but high amount of TP had an adverse effect on microbial growth that may be due to the undesirable effect of high percentages of TP on the texture of the product and the reduced water holding capacity of the sample, thus allowing the microorganisms to grow in the presence of free water. Many researches have been conducted on the antimicrobial effect of different EO for example Nevas *et al.* (2004) examined the antimicrobial properties of various plants against 12 microorganism species, where EOs of oregano, savory, and thyme showed extensive antimicrobial activity and were effective on all 12 bacterial species. *C. botulinum* and *C. perfringens* were the most susceptible microorganisms among the bacteria (Nevas *et al.*, 2004). Oliveria *et al.* (2012) found that the synergy between SN and savory EO in a sample containing 100 ppm of SN at each concentration of savory reduced the population of sausage microorganisms (Oliveira, Carvalho *et al.*, 2012). Silveira *et al.* (2014) found that 0.1g/100g of *Laurus nobilis* essential oil showed a clear effect on pathogens of Tuscan sausage (Silveira *et al.*, 2014).

3.5. Texture hardness

According to Table 3 the analysis of variance (ANOVA) indicated that the linear model was statistically significant and fitted to represent the actual relationship between the hardness of sample and significant variable with a very small P value (<0.0001) and an acceptable coefficient of R^2 (0.9294). Table 3 indicated that there was a negative significant ($p<0.0001$) correlation between TP content and the hardness of sample. There are many articles investigating the effect of plant fibers on the sensory and texture properties of sausages. The results of these investigations are sometimes different due to the type of fiber and its amount as well as process conditions. For example, Yadav *et al.* (2016) observed that the hardness rate would increase if the apple pomace and corn bran were used as fiber in the formulation. However, hardness reduced when the tomato pomace was used. They also observed that hardness decreased by increasing in the amount of TP from 3 to 9%, which was in agreement with our results (Yadav *et al.*, 2016). Garcia *et al.* (2007) observed that with increasing wheat and barley fiber, the amount of hardness would grow significantly(Garcia *et al.*, 2007). Fernandez-Lopez *et al.* (2008) also found that hardness of sausage samples increased in the presence of 0.5 to 2% citrus fiber (Fernández-López *et al.*, 2008). On the other hand, Garcia *et al.* (2002) reported that adding fiber to the sausage would reduce the hardness of the samples and increase their elasticity (Garcia *et al.*, 2002).

3.6. Model optimization and validation

In the optimization process, a range of 0-300 ppm was considered for SN content along with 5-15% for TP, 200-400 ppm for EO, maximum time, minimum residual SN content, L factor within the range of 54.2-66.2, a^*factor of 7.7–10.7, b^* factor of 20.9-25.8, minimum microbial load, and pH and texture without limitation. To test the validity of the model, three experiments were performed under optimal conditions (Table 4).

Table 4. Comparison of predicted and measured dependent variables.

parameters	Predicted	experimental
Sodium nitrite (ppm)	56	56
Tomato powder (%)	5%	5%
Essential oil (ppm)	378	378
Storage time	24	24
Residual nitrite (ppm)	15.23	16.54
L	61	58
a^*	8.9	7.1
b^*	25.7	24.14
Microbial load	85000	90000

As can be seen, the optimal predicted amount of SN (ppm), percentage of TP, savory EO(ppm), and storage time (day) presented by RSM were 56, 5, 378 and, 24, respectively. The software predicted the quality parameters of the sausage by selecting the optimal conditions, as specified in Table 4. In order to confirm the prediction values, the sausage was produced base on the RSM optimized formulation. As it can be observed in Table 4, the experimental results are in good agreement with the predicted values and the reliability of the model was proved. So, it can be used to estimate the test results.

4. Conclusion

The main purpose of this study was to use suitable hurdles in the form of suitable herbal compounds as a part of nitrite replacement in meat products, where its importance will be observed in the problems associated with nitrosamines. In this study, Different levels of nitrite, essential oil of *Satureja bachtiarica* Bunge with high antimicrobial effects, and tomato powder were used. The effects of these factors were investigated on the color, texture, microbial load, pH, and residual nitrite content during storage time. Residual nitrite was strongly dependent on initial nitrite content and storage time and other variables had not any significant effect on it so if the residual nitrite reduction was desired, reduction of initial SN content should be conducted. In order to reduce SN content without any effect on product quality and microbial load the alternatives to SN are essential. TP decreased pH and acted as an antimicrobial hurdle but at higher amount of TP the texture of sausage had not good cohesion and also had an adverse effect on microbial activity. TP also compensated the reduced red color at lower amount of sodium nitrite. EO had a good effect on microbial load and reduction in total count was observed at the last day of storage by increasing EO and had not any effect on texture. After optimization, a formulation with 5% of TP, 378 ppm of *Satureja bachtiarica Bunge* EO, and 56 ppm of SN was suggested by RSM. It was observed that nitrite can be reduced to 56 ppm with the hurdles used in this study, which was a very good value in comparison with EU standard of 150 ppm.

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