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Production of functional sausage using hydrolyzed orange seed proteins as a natural preservative with nitric oxide reduction capability

Narges Mazloomi[1,2]., Atena Ramezani[1,2]., Behnaz Moshrefi[3,4*]

1- Department of Nutritional Sciences, School of Health, Mazandaran University of Medical Sciences, Sari, Iran.

2- The Health of Plant and Livestock Products Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

3- Halal Research Center of IRI, Food and Drug Administration, Ministry of Health and Education, Tehran, Iran

4- Master of Science in Nutrition, Food and Drug Administration, Tehran, Iran.

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*Corresponding Author E-

samira.mazloomi@yahoo.com

ABSTRACT

The defatted flour of orange seeds contains about26%protein. It can be used as a rich and cost-effective source for the production of proteins and peptides of plant origin. In this stage of the research, high-purity protein concentrate was first extracted.Then,using the hydrolyzing enzyme pepsin at an enzyme ratio(1 to3%enzyme to substrate)and a time range(2 to5hours)at a temperature of 30-40°C,the protein from defatted orange seed flour was hydrolyzed. The optimal conditions for producing hydrolyzed proteins with the best nitrite ion inhibitory activity were selected. The optimal treatment under the suggested conditions of temperature,time, and enzyme-to-substrate concentration by the software was produced at a temperature of 39.27°C,time:3.5hours, and a ratio of 2.89%W/W enzyme to substrate,which resulted in the highest activity in the highest activity in inhibiting nitrite ions. This value was 93.46% in inhibiting nitrite ions. In the next stage,the production of fish sausage containing the protein produced from orange seeds in vitro, and then the peroxide index and nitric radical activity of the samples during the storage period was carried out.In general, the use of hydrolyzed protein in fish sausage formulation showed that hydrolyzed protein was significantly able to delay lipid oxidation during storage and samples containing hydrolyzed orange seed protein(increasing its concentration from0.5 to2.5%) significantly increased the nitric oxide radical scavenging activity of the samples.The results showed that the fish sausage formulation containing1.5%hydrolyzed orange seed protein,as a product with high nutritional value and without preservatives, has the potential for industrial production and marketing.

1- Introduction

Nowadays, enzymatic hydrolysis is considered the most common method for modifying the properties of plant and animal proteins. Compared to chemical hydrolysis, this type of hydrolysis is carried out under milder conditions, resulting in less damage to the protein substrate. This method has been used to produce peptides for the enrichment of beverages and food products, patient nutrition, and the production of bioactive compounds (such as inhibitors of enzymes that increase blood pressure and type 2 diabetes, as well as natural antioxidants) in food and pharmaceutical applications. One of the properties of these peptides is their nitric oxide inhibitory (scavenging) activity, as nitric oxide acts as an intermediate compound in the formation of nitrosamines. Nitrate and nitrite salts, which are used as additives to stabilize color and inhibit the growth of certain microorganisms in meat products, are very strong oxidants. Through the reaction of nitrite with secondary and tertiary amines, the carcinogenic compound nitrosamine is formed. However, in the presence of antioxidant compounds in cured meat, these antioxidants react with nitrite much faster than amines, thereby preventing the formation of nitrosamines (1).

Sausage and frankfurter-type products (such as sausages and cold cuts) are among the oldest and most widely consumed ready-to-eat foods, holding a special place in the food basket of consumers worldwide. The per capita consumption of sausages and frankfurters in Iran is approximately 4 kg per citizen (2). A major concern currently associated with these products is the use of nitrate and nitrite as preservatives. Nitrate and nitrite salts are used in meat curing to stabilize the red color, prevent the growth of spoilage and pathogenic microorganisms, and improve flavor. Nitrate is converted to nitrite by starter bacteria present in the meat or in the presence of nitrate and ascorbic acid. Nitrite, in turn, is transformed into nitric oxide under heat, which contributes to the desirable color of the product. However, despite their numerous benefits, high levels of nitrite in meat products are harmful and detrimental from a health perspective. Consequently, the reduction of nitric oxide by sodium nitrite hydration may lead to the formation of nitrous acid, which can react with secondary amines and amino acids present in meat muscles to form nitroso compounds, particularly nitrosamines, ultimately leading to carcinogenesis in body cells. Therefore, the residual levels of these compounds in meat products are of great importance (3). Meshginfar et al. (2016) enzymatically hydrolyzed proteins obtained from sheep viscera and offal, then added them to chicken sausage formulations at concentrations of 700, 1000, and 1250 ppm. They evaluated lipid oxidation by measuring the thiobarbituric acid reactive substances (TBARS) index and peroxide value. The results indicated that increasing the level of hydrolyzed protein significantly reduced lipid oxidation, with effects comparable to those of synthetic antioxidant BHT (1). Izadkhasti et al. (2019) investigated the effects of a mixture of sesame meal and soybean flour on the physicochemical and textural properties of sausages. The findings showed that incorporating sesame meal and soybean flour into German-style sausages could produce a product with acceptable physical, chemical, and textural quality along with higher nutritional value. In that study, the substitution level of 0.06 (0.03 sesame meal + 0.03 soybean flour) was introduced as the optimal treatment (4). Shariat alavi et al. (2022) examined the impact of adding hydrolyzed protein from tomato seeds on the properties of produced sausages. They found that samples containing hydrolyzed protein had lower residual nitrite levels compared to the control sample, and residual nitrite decreased in all samples over time. Additionally, the lightness and redness indices of the product increased with storage time. Therefore, hydrolyzed protein derived from tomato seeds exhibited suitable nitrite-reducing properties and can be used as a functional ingredient in the formulation of meat products (5). The results of the conducted research revealed that orange seeds contain appropriate amounts of minerals, including calcium and zinc, and also exhibits a high content of hydrophobic amino acids. Consequently, defatted orange seed flour can be effectively utilized as a functional additive in food products and as a suitable substrate for producing hydrolyzed proteins (6). Given the richness of defatted orange seed in terms of protein content and amino acid composition, the present study aimed to utilize orange seed as a promising protein source for the production of hydrolyzed proteins possessing nitric oxide (NO) radical scavenging (or reducing) properties. In the subsequent phase, fish sausages incorporating hydrolyzed orange seed protein were produced under laboratory conditions. Then, the peroxide value (as an indicator of oxidative stability) and nitric oxide radical scavenging activity of the samples were evaluated during the storage period. Finally, the overall physicochemical and quality characteristics of the produced product were investigated.

2- Materials and Methods

2-1- Preparation of Hydrolyzed Protein

Enzymatic hydrolysis of proteins obtained from defatted orange seed flour was performed using pepsin enzyme under the optimal temperature and pH conditions of this enzyme. Pepsin was added to the protein solution at enzyme-to-substrate ratios of 1%, 2%, and 3% (w/w). Hydrolysis was carried out for durations ranging from 2 to 5 hours at the optimal temperature (30–40 °C) and at the optimal pH of pepsin. At the end of the hydrolysis period, the enzymatic reaction was inactived by heating the mixture at 85 °C for 10 minutes. Subsequently, the hydrolysates were centrifuged at 12,000 rpm for 15 minutes to remove insoluble residues and excess compounds. The resulting supernatant was collected and stored at –20 °C for subsequent optimization experiments and analyses (7).

2-2- Optimization of the Hydrolysis Process to Achieve the Treatment with the Highest Nitric Oxide Radical Scavenging Activity

In order to optimize the enzymatic hydrolysis process to achieve the maximum antioxidant properties (specifically nitric oxide radical scavenging activity), the Response Surface Methodology (RSM) with a Central Composite Design (CCD) was employed using Design-Expert software. Three independent variables were considered at three coded levels (−1, 0, +1): enzyme-to-substrate concentration (X_1), temperature (X_2), and hydrolysis time (X_3).

The dependent response variable evaluated was the nitric oxide (NO) radical scavenging activity. Accordingly, the software generated a total of 20 experimental runs, including 6 replicates at the center point, which were performed in a completely randomized manner.

Table 1: Levels of independent variables used for optimizing the nitrite inhibition of hydrolyzed orange seed protein.

Levels			independent variables
-1	0	+1	

3	2	1	X1	**Enzyme-to-substrate ratio (%)**
5	3.5	2	X2	**Time (hr)**
40	35	30	X3	**Temperature C)◌̊(**

The regression model developed to predict the desired response (nitric oxide radical scavenging activity) is presented as follows:

Equation 1: Regression model for nitric oxide radical scavenging activity by pepsin enzyme:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 - b_{12}X_1X_2 - b_{13}X_1X_3 - b_{23}X_2X_3 + b_{11}X_1^2 - b_{22}X_2^2 + b_{33}X_3^2$$

Where, Y is the response variable (nitric oxide radical scavenging activity, expressed in actual values), b_0 is the constant, b_1, b_2, b_3 represent the linear effects of the independent variables, b_{11}, b_{22}, b_{33} represent the quadratic effects, b_{12}, b_{13}, b_{23} represent the interaction effects between the variables. The experimental treatments suggested by the Design-Expert software for the enzymatic hydrolysis of orange seed protein using pepsin are presented in the table below, in randomized order.

Table 2: Proposed random treatments for the hydrolysis of orange seed protein by pepsin enzyme

Time (hr)	**Temperature C)◌̊(**	**Enzyme-to-substrate ratio (%)**	**Treatment**
2	30	1	1
5	30	1	2
3.5	30	2	3
2	30	3	4
5	30	3	5
3.5	35	1	6
2	35	2	7
3.5	35	2	8
3.5	35	2	9
3.5	35	2	10
3.5	35	2	11
3.5	35	2	12

3.5	35	2	13
5	35	2	14
3.5	35	3	15
2	40	1	16
5	40	1	17
3.5	40	2	18
2	40	3	19
5	40	3	20

2-3- Measurement of Nitric Oxide Radical Scavenging Activity

60 µL of the hydrolyzed protein solution was mixed with 60 µL of sodium nitroprusside (SNP) in 0.025 M phosphate-buffered saline (PBS). The mixture was then incubated at room temperature for 150 minutes in an incubator. Subsequently, an equal volume (120 µL) of Griess reagent (composed of 1% sulfanilamide, 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride, and 2.5 mL phosphoric acid, made up to 100 mL with distilled water) was added to the mixture. The absorbance was measured spectrophotometrically at 546 nm (8).

The nitric oxide radical scavenging activity was calculated using the following equation:

% Nitric oxide scavenging activity = [(Absorbance of control – Absorbance of sample) / Absorbance of control] × 100

Where,absorbance of control: absorbance of the reaction mixture without the sample (hydrolyzed protein)., absorbance of sample: absorbance of the reaction mixture containing the hydrolyzed protein.

2-4- Peroxide Value

To determine the peroxide value, 3 g of the sample was weighed and mixed with 30 mL of acetic acid-chloroform solution (Chloroform: Acetic acid ratio of 2:3). Then, 0.5 mL of saturated potassium iodide solution was added, and the mixture was shaken for 1 minute. Subsequently, 30 mL of distilled water was added to the mixture and shaken again. The resulting solution was titrated with 0.01 N sodium thiosulfate solution until a light yellow color appeared. At this point, 0.5 mL of 1% starch solution was added as an indicator to produce a blue color. Titration was continued until the blue color completely disappeared.

The peroxide value was calculated using the following equation: (9)

$$\text{Peroxide Value } (\text{mEq}/Kg) = \frac{S \times M \times 1000}{m}$$

S = volume of sodium thiosulfate consumed (mL)

M = molarity of sodium thiosulfate solution (0.01 N)

m = weight of the sample (g)

The results were expressed as milliequivalents of active oxygen per kilogram of sample (meq O_2/kg).

2-5- Production of Fish Sausage Containing Hydrolyzed Orange Seed Protein

To produce the fish sausages, the formulation reported by Hajfathalian et al. (2019) was used with slight modifications. The general sausage formulation consisted of the following ingredients (w/w): ground Marlin fish meat 70%, Sunflower oil 5%, Water and Ice 15%, Starch and Gluten 5%, Salt and Spices 2%, and Onion Powder 3%. The formulation components were weighed according to the specified ratios and mixed with half of the total water and ice in a bowl cutter. Subsequently, the remaining half of the water and ice along with the hydrolyzed protein (prepared under the optimized conditions exhibiting the highest nitric oxide radical scavenging activity) was added to the mixture at concentrations of 0%, 0.5%, 1.5%, and 2.5% (w/w) (Table 3). A positive control sample containing 0.15% ascorbic acid (vitamin C) was also prepared. The sausages were heat-treated at 80 °C for 1 hour, followed by immediate cooling in chilled water (8 °C). After the internal temperature of the sausages reached room temperature, they were transferred to a refrigerator at 4 °C and stored for 30 days.

The antioxidant properties (peroxide value) and nitric oxide radical scavenging activity of the samples were evaluated at storage intervals of 0, 5, 10, 15, 20, 25, and 30 days (10).

Table 3: Different treatments of fish sausage

Sample	Hydrolyzed Proteins (%)	(%) Vit C
Blank	0	0
Treatment1	0.5	0
Treatment2	1.5	0
Treatment3	2.5	0
Treatment4	0	1.5

2-6- Statistical Analysis

To optimize the enzymatic hydrolysis process of orange seed protein concentrate, Response Surface Methodology (RSM) with a Central Composite Design (CCD) was employed, and the contour and response surface plots were generated using Design-Expert software (version 13, Stat-Ease Inc., Minneapolis, USA). In this study, the Design-Expert software was utilized to implement the Response Surface Methodology experimental design. The design was constructed as a Central Composite Design consisting of a total of 20 experimental runs, which included: six replicates at the center point, seven axial points, and seven factorial points.

The independent variables investigated were: Hydrolysis time (X_3: 2–5 hours), Enzyme-to-substrate ratio (X_1: 1–3%), Hydrolysis temperature (X_2: 30–40 °C). The dependent variable was the nitric oxide (NO) radical scavenging activity.

Statistical analysis of the other experimental results in this study was performed using a factorial experimental design. Mean comparisons were conducted using Duncan's multiple range test at a 95% confidence level ($p < 0.05$). The present research was registered as project number 56 at Halal Research Center of IRI.

3- Results and Discussion

3-1- Chemical Composition

The moisture, ash, fat, protein, and carbohydrate contents of the initial defatted orange seed flour and the orange seed protein concentrate are presented in Table 3. The results indicated that the defatting process led to a significant reduction in the fat content of the protein concentrate (from 43.38% to 5.46%). The protein content of the initial defatted meal flour was 22.47%, whereas the obtained protein concentrate contained 75.12% protein. As shown in the table, the moisture content of the defatted orange seeds was 7.13 ± 0.52%. This value is higher than the previously reported value of 5.50 ± 0.8% in previous studies. The fat and ash contents of the defatted orange seeds (dry weight basis) were 5.46 ± 0.15% and 2.66 ± 0.17%, respectively. These values are lower for fat (compared to the reported 54.2 ± 0.12%) and higher for ash (compared to the reported 2.5 ± 0.23%) than those documented in previous studies (5). The protein content of defatted flour was measured as 22.47 ± 3.51%, while that of the protein concentrate was 75.12 ± 1.41%. Based on these results, the defatting process resulted in a higher protein yield, and the defatting treatment significantly reduced the fat content of the protein concentrate. The differences observed in the chemical composition of the samples used in the present study compared to previous reports can be attributed to variations in the orange variety, geographical origin and differences in the defatting and protein isolation processes employed (2).

Table 3: Chemical composition of defatted orange seed meal and protein concentrate

protein concentrate	defatted orange seed meal	
75.12 ±1.41	22.47 ± 3.51	Protein (N×6.25)
5.46 ± 0/15	43.38 ± 0/58	Fat
8.79 ± 0/17	7.13 ± 0/52	Moisture
1.6 ± 0/23	2.66 ±0/17	Ash
9.03	24.36± 0/12	Carbohydrate

*All data were expressed as a mean of 3 replications ± standard deviations.
* Values were reported based on dry weight.

3-2- Optimization of the Process to Achieve the Treatment with the Highest Nitrite (Nitric Oxide) Radical Scavenging Activity

Table 4: Process optimization to achieve the treatment with the highest nitrite inhibition power

treatments	Enzyme concentration (%)	Temperature ° (C)	Time (hr)	Nitric oxide reducing power (%)
1	1	30	5	90.18
2	3	35	3.5	92.04
3	3	30	2	90.52
4	2	35	5	93.57
5	3	30	5	94.41
6	3	40	2	94.75
7	2	35	3.5	93.23
8	2	35	2	90.52
9	1	30	2	91.20
10	1	40	2	89.84
11	2	35	3.5	90.52
12	3	40	5	90.69
13	2	35	3.5	91.20
14	2	35	3.5	90.86
15	1	35	3.5	91.37
16	2	30	3.5	87.47
17	2	40	3.5	95.26
18	1	40	5	94.58
19	2	35	3.5	92.04
20	2	35	3.5	91.03

3-3- Evaluation of Nitrite Ion Scavenging Activity

Equation 3 was developed based on the regression coefficients and their significance for the desired response (nitrite ion scavenging activity) as follows:

$$Y = +91.50 + 0.66A + 1.13B + 0.52C - 0.27AB - 0.49AC - 0.32BC + 0.52A^2 - 0.16B^2 + 0.18C^2$$

Figure 1 illustrates the three-dimensional response surface showing the effect of time and temperature on nitrite ion scavenging activity. As shown in the figure, increasing the hydrolysis temperature from 30 to 40°C significantly enhanced the nitrite scavenging capacity of the obtained hydrolysates from 71.85% to 93.04%. On the other hand, increasing the hydrolysis time from 1 to 5 hours also improved the nitrite scavenging ability of orange seed protein hydrolysate from 65.85% to 88.72%. Overall, the highest nitrite ion scavenging activity (92.95%) was achieved at a constant enzyme-to-substrate ratio of 2%, hydrolysis time of 1.02 hours, and temperature of 38.87°C.

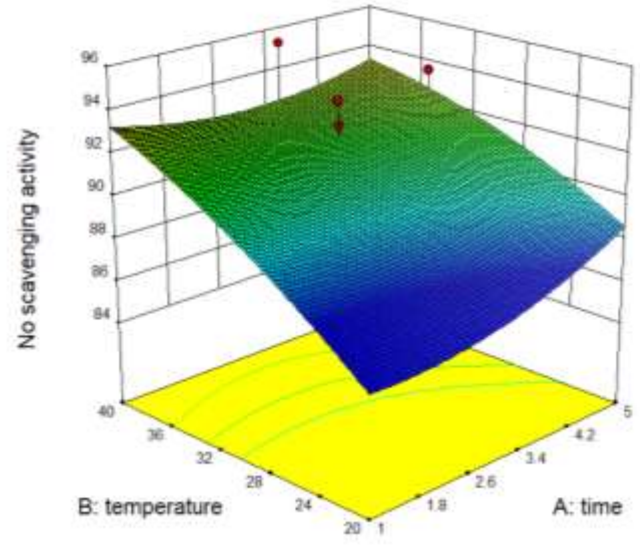

Fig 1: 3D graph for the effect of temperature and the hydrolysis time on the nitric oxide reducing power when concentration of pepsin enzyme was at optimum value (% 2).

Figure 2 illustrates the three-dimensional response surface showing the effect of hydrolysis time and enzyme-to-substrate ratio on nitrite ion scavenging activity. As shown in the figure, increasing the pepsin concentration from 1% to 3% resulted in a significant enhancement of the nitrite scavenging capacity of orange seed protein hydrolysate, from 90.72% to 94.72%. On the other hand, hydrolysis time also exerted a significant effect on the nitrite scavenging ability of the obtained hydrolysates; increasing the hydrolysis time from 1 to 5 hours improved the scavenging capacity of orange seed protein hydrolysate from 90.68% to 92.65%. Overall, at a constant temperature of 35°C, the highest nitrite ion scavenging activity (94.90%) was achieved at a hydrolysis time of 1.01 hours and an enzyme-to-substrate ratio of 2.86%.

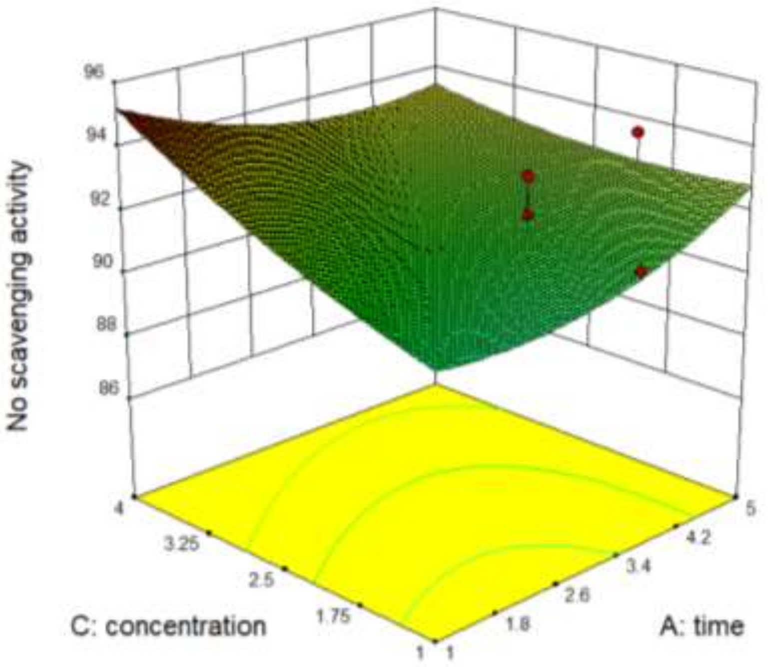

Fig 2: 3D graph for the effect of concentrationof pepsin enzyme and the hydrolysis time on the nitric oxide reducing power when the hydrolysis temperature was at optimum value (35°C).

Figure 3 illustrates the three-dimensional response surface showing the effect of temperature and enzyme-to-substrate ratio on nitrite ion scavenging activity. As shown in the figure, increasing the pepsin enzyme-to-substrate ratio from 1% to 3% resulted in an enhancement of the nitrite ion scavenging activity from 85.33% to 90.23%. On the other hand, increasing the hydrolysis process temperature from 20 to 40°C also had a positive effect on the nitrite scavenging capacity of orange seed protein hydrolysate, raising it from 85.46% to 95.31%. Overall, at a constant hydrolysis time of 3.5 hours, the highest nitrite ion scavenging activity (93.46%) was achieved at a temperature of 39.27°C and an enzyme-to-substrate ratio of 2.89%.

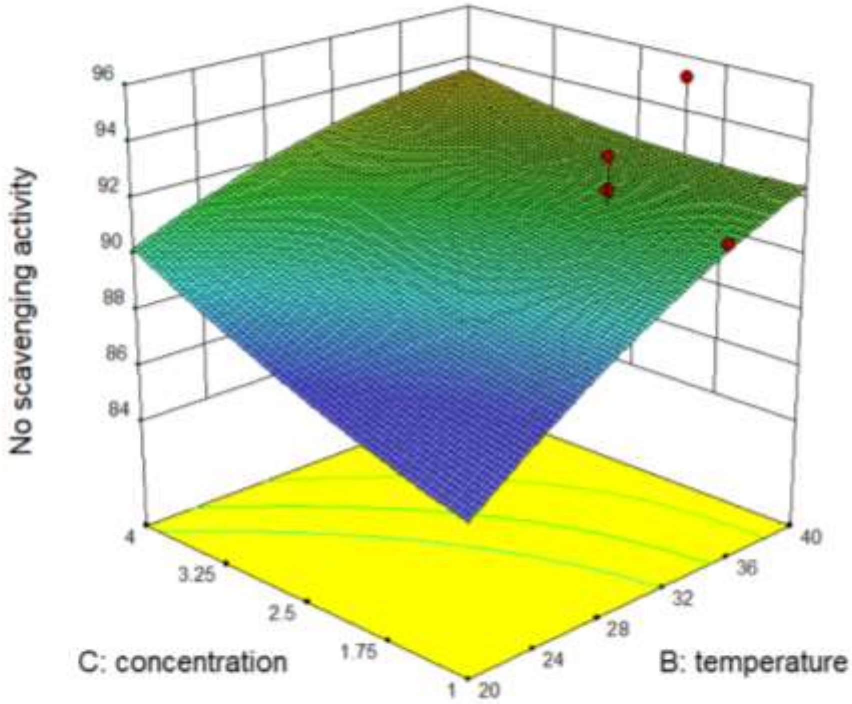

Fig 3: 3D graph for the effect of concentrationof pepsin enzyme and the hydrolysis temperature on the nitric oxide reducing power when the hydrolysis time was at optimum value (3.5 hr).

The optimal treatment under the suggested conditions of temperature, time, and enzyme-to-substrate ratio (as proposed by the software) was prepared. Antioxidant assays, including DPPH radical scavenging activity, ferric reducing power and nitrite ion scavenging activity were performed on the optimal treatment to validate the values predicted by the software. Finally, the resulting supernatant was freeze-dried using a freeze dryer and stored at −18°C.

In the studies conducted by Zakeri et al., (2019) and Shariat alavi et al., (2019), it was found that pumpkin seed protein hydrolysate and tomato seed protein hydrolysate, respectively, exhibit considerable nitric oxide scavenging capacity. Therefore, they can serve as natural compounds with high nitric oxide inhibitory properties as alternatives to synthetic preservatives in food products, including meat products. Furthermore, these studies revealed that increasing the enzyme concentration leads to an enhancement in the inhibitory activity (11, 2).

In the study conducted by Zhonggao et al., (2005), the nitrite radical scavenging activity of black berry extract was investigated. The results demonstrated that the nitrite radical trapping capacity was negligible at low concentrations and gradually increased with higher extract concentrations (12). In another study, Nikkhah et al,. (2011) evaluated the nitric oxide scavenging activity of Anthocyanins from three different blackberry species. Their findings indicated that the inhibitory capacity increased concomitantly with increasing concentrations of the anthocyanin-rich blackberry extracts (13).

3-4- Peroxide Value

Fish meat contains a high amount of unsaturated lipids , therefore highly susceptible to lipolysis and auto-oxidation. Exposure of fish meat to atmospheric oxygen leads to lipid oxidation and the formation of hydroperoxides. The peroxide value is an indicator of the primary oxidation stage of lipids (14). Changes in the peroxide value of fish sausages during 30 days of refrigerated storage are presented in Figure 4. Overall, the peroxide value of all samples increased significantly ($p < 0.05$) throughout the storage period. The control sample exhibited the highest peroxide value, rising from 5.6 meq O_2/kg on day 0 to 32.71 meq O_2/kg on day 30 of storage. On the other hand, the addition of protein hydrolysate at concentrations of 1.5% and 2.5% significantly reduced the rate of increase in peroxide value. Notably, the peroxide value of the sample containing 0.5% protein hydrolysate showed no significant difference compared to the control sample ($p > 0.05$). At the end of the storage period, among the samples containing protein hydrolysate, the lowest peroxide value (19.55 meq O_2/kg) was observed in treatment 3, which contained 2.5% protein hydrolysate. Furthermore, the peroxide value of the control sample and treatment 1 decreased on day 20 of storage, while that of samples 4, 3, and 2 decreased on day 30. This decreasing trend may be attributed to the decomposition of hydroperoxides and the subsequent formation of secondary oxidation products such as aldehydes and ketones (15). Comparison of the treatment containing Ascorbic acid (vitamin C), (treatment 4) with the treatments containing protein hydrolysate, treatment 4 exhibited the lowest peroxide value throughout all storage days. This indicates that, despite the considerable antioxidant potential of orange seed protein hydrolysate, this compound was not as effective as ascorbic acid in controlling lipid oxidation in fish sausages. The precise protective mechanism of protein hydrolysates in delaying lipid oxidation has not yet been fully elucidated. However, it has been reported that natural antioxidant compounds, such as protein hydrolysates, react with lipid peroxyl and lipid alkoxyl radicals, thereby preventing their further decomposition (14). Studies have demonstrated that the molecular size, composition, and amino acid sequence of protein hydrolysates play a critical role in determining their antioxidant activity (14). Similar to these findings, Hajfathalian et al., (2019) reported that the peroxide value of fish sausages containing protein hydrolysate from common carp (Cyprinus carpio) spleen increased during the storage period; however, this increasing trend was significantly lower in the samples containing protein hydrolysate compared to the control sample (10). Additionally, Feng et al., (2020) produced fish sausages incorporated with alpha-tocopherol nanoemulsion and reported that the peroxide value of the sausage samples significantly increased over 16 days of storage. Nevertheless, the addition of alpha-tocopherol nanoemulsion at a concentration of 500 mg/kg significantly reduced the rate of increase in peroxide value (16).

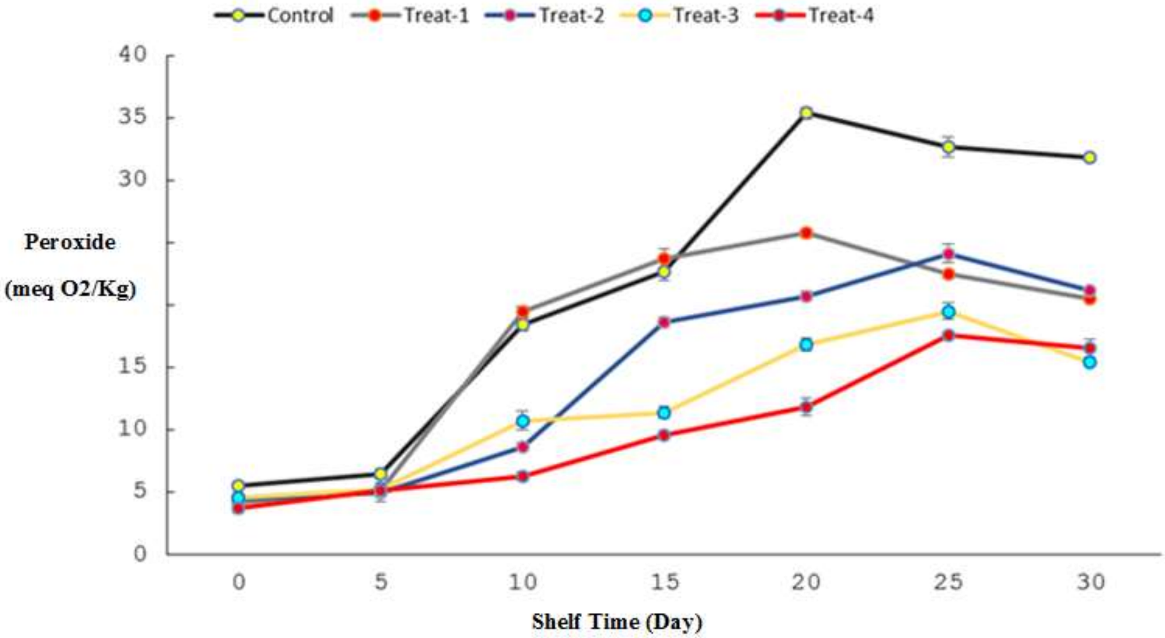

Fig 4: Peroxide index of fish sausage containing different concentrations of hydrolyzed orange seed protein during storage. **Control:**Sample without hydrolyzed protein., **Treat 1:** Sample with 0.5% of hydrolyzed protein., **Treat 2:** Sample with 1.5% of hydrolyzed protein., **Treat 3:** Sample with 2.5% of hydrolyzed protein., **Treat 1:** Sample with 1.5% of Vit C.

3-5- Nitric Oxide Radical Scavenging Activity During Storage

Figure 5 illustrates the changes in nitric oxide radical scavenging activity of the sausage samples during the storage period. At the beginning of the storage period, the lowest nitric oxide radical scavenging activity was observed in the control sample and treatment 1, with values of 34.18% and 40.42%, respectively. There was no significant difference between these two treatments ($p > 0.05$). On the other hand, the highest nitric oxide radical scavenging activity belonged to treatment 3, with a value of 90.17%. Overall, the nitric oxide radical scavenging activity of all samples exhibited a decreasing trend throughout the storage period. This decline was more pronounced in sample 4 (containing vitamin C); this may be attributed to the greater sensitivity of vitamin C compared to protein hydrolysate to environmental factors such as light, oxygen, and others (17). In the case of samples containing orange seed protein hydrolysate, increasing its concentration from 0.5% to 2.5% significantly enhanced the nitric oxide radical scavenging activity of the samples. Consequently, at the end of the storage period, the highest antioxidant capacity belonged to sample 3 with 62.73%, while the lowest nitric oxide radical scavenging activity was observed in sample 1 with 14.2%. The negligible but non-negligible nitric oxide radical scavenging activity of the control sample can be attributed to the antioxidant potential of the spices used in the sausage formulation, owing to their phenolic and flavonoid compounds. The increase in nitric oxide radical scavenging activity of the sausages with higher concentrations of protein hydrolysate is likely due to the inherent antioxidant potential of the orange seed protein hydrolysate.

Similar to these findings, Tawali et al., (2023) reported that the incorporation of Snakehead (Channa striata) Gelatin into beef sausages resulted in samples with considerable antioxidant capacity in terms of DPPH free radical scavenging activity. This antioxidant capacity significantly increased with higher concentrations of gelatin. On the other hand, they also reported a significant decrease in antioxidant capacity during 28 days of refrigerated storage (18).

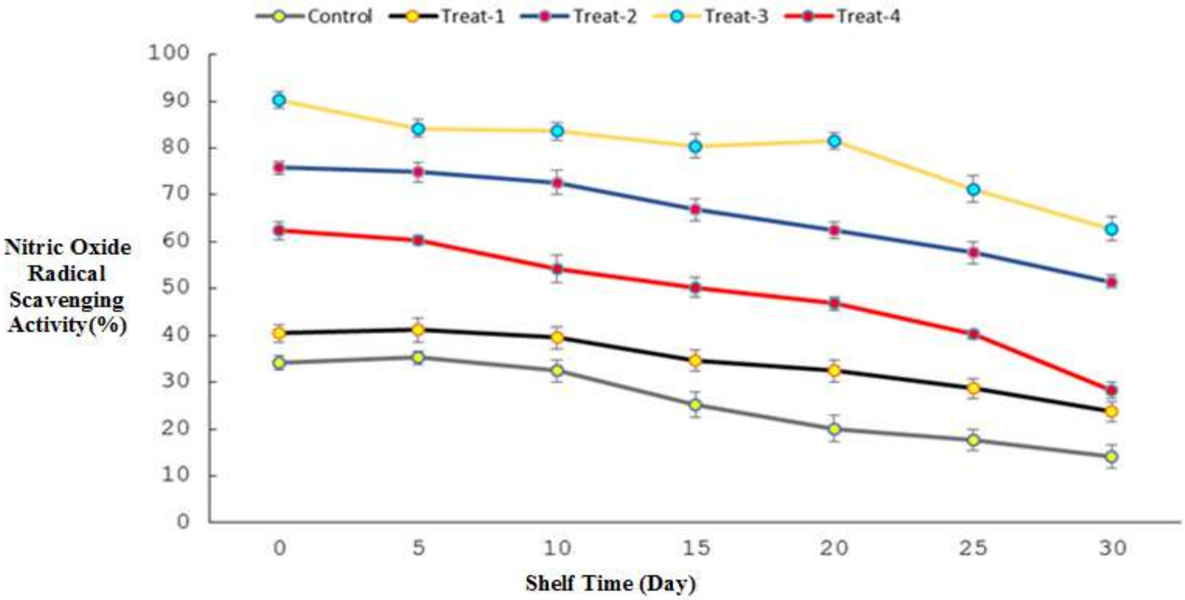

Fig 5: Changes in nitric oxide radical inhibition of fish sausage samples containing different concentrations of hydrolyzed orange seed protein during storage. **Control:**Sample without hydrolyzed protein., **Treat 1:** Sample with 0.5% of hydrolyzed protein., **Treat 2:** Sample with 1.5% of hydrolyzed protein., **Treat 3:** Sample with 2.5% of hydrolyzed protein., **Treat 1:** Sample with 1.5% of Vit C.

4- Conclusion

The results obtained from the optimization of hydrolysis conditions for producing protein hydrolysates with nitrite ion scavenging activity using pepsin enzyme and the response surface methodology demonstrated that the optimal conditions for hydrolyzing orange seed protein, with the highest nitrite ion scavenging activity, were achieved at a constant hydrolysis time of 3.5 hours, temperature of 39.27°C, and enzyme-to-substrate ratio of 2.89%. Under these conditions, a nitrite ion scavenging activity of 93.46% was obtained. To confirm the conditions suggested by the mathematical equation, additional experiments (in three replicates) were conducted under the predicted conditions. The experimental values were highly consistent with those predicted by the model, confirming these as the optimal conditions for producing protein hydrolysate with nitrite ion scavenging capacity from defatted orange seed flour. Furthermore, the incorporation of protein hydrolysate in the fish sausage formulation demonstrated that this hydrolysate was significantly capable of delaying lipid oxidation during the storage period. This is evidenced by the fact that samples containing protein hydrolysate exhibited lower peroxide values compared to the control sample. Moreover, increasing the concentration of orange seed protein hydrolysate from 0.5% to 2.5% significantly enhanced the nitric oxide radical scavenging activity of the samples. Overall, the results indicate that fish sausage formulated with 1.5% orange seed protein hydrolysate can be produced as a high-nutritional-value product free from synthetic preservatives, possessing the potential for industrial-scale production and market introduction. The present study demonstrated that wastes from the orange juice industry can serve as an accessible and novel protein source for the production of protein hydrolysates. Considering the potent nitric oxide radical scavenging activity of these hydrolysates, they possess considerable potential for use as a natural additive in the production of meat products.

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Author Contributions

All activities were carried out by the author.

Competing Interests

The author confirms that he / she has no financial conflicts of interest or competing interests in this study

5- References

[1] Meshginfar, N., Sadeghimahonak, A. R., Ghorbani, M., Aalami, M. (2016). Effects of protein hydrolysate sheep visceral on oxidative stability of soybean oil and chicken sausage. Journal of Food Processing and Preservation ISSN. 1745-4549.

[2] Zakeri, K., Ghorbani, M., Sadeghi Mahoonak, A. R., Moayedi, A., Maghsoudlou, Y. (2019). Determination of Optimum Conditions for the Production of Peptides with Antioxidant and Nitric Oxide Inhibition Properties from Protein Hydrolysis of Pumpkin Seed Meals Using Pepsin Enzyme. Iranian Journal of Nutrition Sciences & Food Technology. 14(3), 14-50.

[3] Karimzadeh, L., Koohdani, F., Mahmoudi, M., Safari, F., Babaee, Z. (2010). Determination of nitrate and nitrite residues in smoked Caspian Kutum, Rutilus frisii kutum and Mullet, Liza auratus in the north of Iran. World Journal Fish and Marine Science. 2(1), 62-65.

[4] Izadkhasti Z, Fazel M., Abbasi H. (2019). Studying the effect of sesame meal and soybean flour mixture on the physicochemical and textural properties of sausage. Food Technology & Nutrition. 16 (4), 33-45.

[5] Shariat alavi M., Sadeghi Mahoonak AR., Ghorbani M., Alami M., Mohamadzade J. (2022). Study on the effect of tomato seed protein hydrolyzate on the characteristics of susage. Journal of food science and technology. 121 (18), 301-313.

[6] Mazloomi S. N., Sadeghi Mahoonak A., Mora L., Ghorbani M., Houshmand GH., Toldrá F. (2021). Pepsin Hydrolysis of Orange By-Products for the Production of Bioactive Peptides with Gastrointestinal Resistant Properties. Foods. 10, 679. https://doi.org/10.3390/foods10030679.

[7] Matsuoka T., Kawashima T., Nakamura T., Kanamaru Y., Yabe T. (2012). Isolation and characterization of proteases that hydrolyze royal jelly proteins from queen bee larvae of the honeybee, Apis mellifera. Apidologie.43, 685-697.

[8] Tsai P. J., Tsai T. H., Yu C. H., Ho S. C. (2007). Comparison of No-scavenging and NO-suppressing activity of different herbal teas with those of green tea. Food Chemistry. 103,181-187.

[9] Feng X., Tjia J.Y.Y., Zhou Y., Liu Q., Fu C., Yang H. (2020). Effects of tocopherol nanoemulsion addition on fish sausage properties and fatty acid oxidation. LWT. 118,108737.

[10] Hajfathalian M., Jorjani S. Ghelichi S. (2020). Characterization of fish sausage manufactured with combination of sunflower oil and fish oil stabilized with fish roe protein hydrolysates. Journal of Food Science and Technology. 57(4), 1439-1448.

[11] Shariat alavi M. 1, Sadeghi Mahoonak A. R., Ghorbani M., Alami M., Mohamadzade J.

(2019). Determination of Optimum Conditions for Production of Hydrolyzed Protein with Antioxidant Capability and Decrease of Nitric Oxide from Tomato Wastes by Alcalas. Journal of food science and technology. 84 (15), 137-151.

[12] Zhonggao C., Felgines O., Texier C., Besson DJ., Liu J., Wang S. (2005). Antioxidant activities of total pigment extract from blackberries. Food Technology and Biotechnology. 43(1), 97-102.

[13] Nikkhah A., Khayami M., Heidari R. (2011). Evaluation of nitric oxide scavenging activity of anthocyanins from black berry (Morus nigra L.), strawberry (Fragaria vesca L.) and berry (Morus alba L. Var. nigra) extracts. Scientific & Research Journal of Iranian Medicinal & Aromatic Plants. 25(1), 120-128. [in Persian]

[14] Vanitha M., Dhanapal K., Vidya Sagar Reddy G. (2015). Quality changes in fish burger from Catla (Catla Catla) during refrigerated storage. Journal of Food Science and Technology. 52, 1766-1771.

[15] Intarasirisawat R., Benjakul S., Visessanguan W., Wu J. (2014). Effects of skipjack roe protein hydrolysate on properties and oxidative stability of fish emulsion sausage. LWT-Food Science and Technology. 58(1), pp.280-286.

[16] Feng X., Tjia J.Y.Y., Zhou Y., Liu Q., Fu C., Yang H. (2020). Effects of tocopherol nanoemulsion addition on fish sausage properties and fatty acid oxidation. LWT, 118, 108737.

[17] Çakmakçi S., Turgut T. (2005). Influence of different light sources, illumination intensities and storage times on the vitamin C content in pasteurized milk. Turkish Journal of Veterinary & Animal Sciences. 29(5), 1097-1100.

[18] Tawali A.B., Said M.I., Sari S.F., Anwar L.O., Nurdin I.N., Said A., Tamtama A., Auza F.A., Zzaman W., Jeinie M.H., Rahman M.N.A. (2023). Characteristics of the Beef Cheek Meat-Based Sausage Added with Snakehead (Channa striata) Gelatin. International Journal of Food Science. 102, 54-68.

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تولید سوسیس فراسودمند با استفاده از پروتئین های هیدرولیز شده هسته پرتقال به عنوان نگهدارنده طبیعی و با قابلیت کاهندگی نیتریک اکسید

نرگس مظلومی[۱,۲]؛ آتنا رمضانی[۱,۲]؛ بهناز مشرفی *[۳,۴]

۱- استادیار گروه تغذیه، دانشکده بهداشت دانشگاه علوم پزشکی مازندران، ساری، ایران

۲- مرکز تحقیقات سلامت فرآورده‌های گیاهی و دامی، دانشگاه علوم پزشکی مازندران، ساری، ایران

۳- مرکز تحقیقات حلال جمهوری اسلامی ایران، سازمان غذا و دارو، وزارت بهداشت، درمان و آموزش پزشکی، تهران، ایران

۴- کارشناس ارشد علوم تغذیه سازمان غذا و دارو، تهران، ایران

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* مسئول مکاتبات:

samira.mazloomi@yahoo.com

چکیده

آرد چربی گیری شده هسته پرتقال، حدود ۲۶درصد پروتئین دارد و می‌تواند به عنوان منبع غنی و مقرون به صرفه برای تولید پروتئین‌ها و پپتیدهایی با منشاء گیاهی مورد استفاده قرار گیرد. در این مرحله از پژوهش ابتدا استخراج کنسانتره پروتئین با خلوص بالا صورت گرفت، سپس با استفاده از اثر آنزیم هیدرولیز کننده پپسین در نسبت آنزیم (۱تا ۳٪)و بازه زمانی (۲تا ۵ساعت)در دمای ۴۰-۳۰درجه سانتیگراد، پروتئین آرد چربی گیری شده هسته پرتقال هیدرولیز شد و شرایط بهینه برای تولید پروتئین‌های هیدرولیز شده دارای بهترین فعالیت مهارکنندگی یون نیتریت انتخاب شد. تیمار بهینه در شرایط دمایی، زمانی و نسبت غلظت آنزیم به سوبسترا که توسط نرم افزار پیشنهاد شده بود،(دمای۳۹/۲۷درجه سانتی‌گراد،زمان:۳/۵ساعت و نسبت ۲/۸۹درصد وزنی-وزنی آنزیم به سوبسترا با بیشترین فعالیت بازدارندگی یون نیتریت) تولید گردید که این مقدار ۹۳/۴۶درصد در قدرت بازدارندگی یون نیتریت بود. در مرحله بعدی تولید سوسیس ماهی حاوی پروتئین هیدرولیز شده هسته پرتقال در محیط آزمایشگاهی و سپس اندیس پراکسید و فعالیت مهار رادیکال نیتریک اکسید نمونه ها طی دوره نگهداری انجام شد. بطورکلی کاربرد پروتئین هیدرولیز شده در فرمولاسیون سوسیس ماهی نشان داد که پروتئین هیدرولیز شده به‌طور معنی‌دار قادر به تاخیر اکسیداسیون لیپیدی طی دوره نگهداری میباشد و نیز نمونه‌های حاوی پروتئین هیدرولیز شده هسته پرتقال(افزایش غلظت آن از ۰/۵به ۲/۵درصد)به‌طور معنی‌داری باعث افزایش فعالیت مهار رادیکال نیتریک اکسید نمونه‌ها شد.نتایج حاصل نشان داد که فرمولاسیون سوسیس ماهی حاوی۱/۵درصد پروتئین هیدرولیز شده هسته پرتقال به‌عنوان یک محصول با ارزش غذایی بالا و بدون نگهدارنده قابلیت تولید صنعتی و عرضه به بازار را دارد.