



Hydrolyzed collagen and cranberry powder in cooked sausages: Optimization using RSM

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Abstract:

Functional components from animal by-products fortified by plant-based functional ingredients constitute a contemporary strategy that is congruent with the objectives of clean-label reformulation.

This research examined a cooked sausage matrix fortified with hydrolyzed collagen (5–15%) and cranberry powder (1–3%). The sausages were evaluated in terms of hardness, cohesiveness, color stability, total antioxidant capacity (FRAP), and sensory profile. A full 3 × 3 design incorporated second-order response surfaces and composite desirability. It made it possible to quantify the effects of factors via response surface methodology.

Cranberry powder predominantly enhanced color stability and FRAP across the factor space, while hydrolyzed collagen primarily modulated hardness, cohesiveness, and other sensory attributes. Cranberry powder exhibited substantial enhancements in pigment protection and redox status. This effect was attributable to polyphenols, which functioned through electron donation and metal chelation, thereby retarding the oxidation of myoglobin and lipids in cooked sausages. As a result, peptide-mediated softening occurred at higher amounts of hydrolyzed collagen, and maximum cohesion was reached at moderate amounts.

The optimal combination, according to overall desirability profiles, was hydrolyzed collagen – 10.0% and cranberry powder – 2.0%. At this specific point, the responses were as follows: hardness – 3.746 g/mm², cohesiveness – 81.92%, color stability – 89.62%, FRAP – 552.75 a.u., and sensory profile – 4.10 points. The predictability at the optimum level was high, with a mean relative error of 0.61%.

Keywords: Collagen hydrolysate, cranberry powder, cooked sausage, response surface methodology, functional variables, antioxidant capacity (FRAP), color stability, sensory profile

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INTRODUCTION

The last decade of studies concerning upgraded collagen-rich animal by-products as functional ingredients for meat systems focused on circular bioeconomy and clean-labeling. Enzymatic hydrolysates of collagens are made of skin, tendons, and other parts. They show techno-functional potential in processed meat systems, which leads to the enhancement of the protein matrix, water-binding capacity, yield, and even antioxidant capacity [1–3]. For example, adding these hydrolysates to

cooked pork improved the yield and textural characteristics of the final product. Gelatins hydrolyzed from fish skin improved the emulsion stability and textural properties of beef sausages. However, such studies are often limited to a single factor and small concentration ranges, which means further research is needed to apply these results to commercial sausage formulations [4–6].

Cranberries (*Vaccinium macrocarpon* Aiton) are a potential plant antioxidant for meat formulations. The powder and extracts of cranberries are abundant in

such antioxidants as ascorbic acid, tocopherols, and proanthocyanidins that inhibit lipids peroxidation and improve the color stability of meat products [7]. Fermented and cooked models with berry extracts are low in thiobarbituric acid reactive substances but possess good color retention. Cranberries remain within the list of efficacious botanical material within the scope of “fruit extracts in meat” discussion. Most studies use *in vitro* environment and/or reduced patty models. Some focus on the oxidation aspects and textural changes rather than appearance of sliced cooked sausages [8–10].

Very limited evidence supports the combined utilization of animal protein hydrolysates and plant polyphenols in a single meat system. The majority of experiments involve testing only one of the components, either hydrolysates or botanical antioxidants. Very few investigate the dose-response behavior of their joint interaction in real meat product formulations [11, 12]. We argue that this issue is quite significant since peptide fragments from proteins and polyphenols can form complexes non-covalently via hydrogen bonding or by hydrophobic interactions and, under oxidation conditions, through covalent linkages. These complexes alter gelation, water mobility, and color changes. Literature reviews on interactions between phytophenol and muscle-protein report that, depending on pH, ionic strength, and phenolic profile, such complexes may stiffen or weaken gels and thus affect the myoglobin redox cycling, which is related to color stability [13, 14]. Lack of explicit two-factor designs means that for cooked sausages the issue of synergistic (antioxidant protection of peptide-structured gels) or antagonistic (polyphenol-induced weakening of the protein network) effects remains unresolved.

Response surface methodology (RSM) is considered a traditional approach in meat research for single outcome optimization, such as yield, texture, or oxidation [15, 16]. However, the multi-response optimization that combines RSM with the Derringer–Suich desirability framework is very rarely applied in meat formulation studies. In places where this technique is employed, it mostly focuses on safety or process metrics rather than the concurrent optimization of texture, color, and antioxidant capacity in sausages. This methodological gap exposes the industry to the lack of transparent targets that link statistical optima with technological acceptability.

The task remains to figure out these combined levels of hydrolyzed collagen (valorized from animal by-products) and cranberry powder within realistic processing ranges. These ranges are to maximize antioxidant capacity and color stability while maintaining hardness, cohesiveness, and overall sensory characteristics of cooked sausages at technologically acceptable levels. Solving this problem necessitates a two-factor RSM study combined with Derringer–Suich multi-response desirability with experimentally validated targets for all responses.

The aim of the research was to quantify and model the combined effects of hydrolyzed collagen and cranberry powder on the texture, color stability, antioxidant

capacity, and sensory properties of cooked sausages. We achieved this aim by constructing and analyzing statistically valid response-surface models and determining an optimal sausage formulation using the Derringer–Suich multi-response desirability functions.

STUDY OBJECTS AND METHODS

The research featured the formulation and processing of cooked sausage fortified with two functional components, i. e., hydrolyzed collagen and cranberry powder. To obtain hydrolyzed collagen, fetlock joints (80–100 g) were cleaned, washed, and cut. After defatting in water ($t = 60\text{--}65^\circ\text{C}$; $\tau = 45\text{--}50$ min), the material was cooled down to 45°C and hydrolyzed enzymatically ($t = 45^\circ\text{C}$; $\tau = 24$ h) using 1% of proteolytic enzyme preparation BLT-7. Its proteolytic activity was 207.2 ± 4.8 U/mL, and it was obtained from *Bacillus licheniformis* (National Center for Biotechnology, Astana, Kazakhstan). The supernatant was heated ($t = 95 \pm 2^\circ\text{C}$; $\tau = 30$ min) to inactivate enzymes. The hydrolysate was spray-dried (inlet $t = 135\text{--}140^\circ\text{C}$; outlet $t = 85\text{--}90^\circ\text{C}$) in a mini spray dryer (B-290, Buchi, Switzerland) for subsequent use. Cranberries were dried ($40\text{--}45^\circ\text{C}$ for 12–16 h) in a dehydrator (FD1104, Redmond, Russia) in order to preserve polyphenols and vitamin C, then homogenized using a cutting mill Grindomix GM 200, Retsch (Germany).

The cooked sausages consisted of skinless deboned broiler chicken breast (45%), lean beef with minimal visible fat and connective tissue (45%), and bovine visceral fat (10%). Only two formulation factors, i. e., hydrolyzed collagen and cranberry powder, were variables. All other components and processing parameters remained constant to isolate formulation effects. The formulation components included 2% of salt, 0.005% sodium nitrite, and 0.08% ground nutmeg. The processing parameters were as follows: cutter time – 10 min, cooking profile – 55 min at 90°C and 45 min at 80°C . The last stage presupposed chilling in a cold-water bath until the core temperature reached $12\text{--}15^\circ\text{C}$. The storage implied under standard laboratory conditions, i. e., in the dark at $2 \pm 2^\circ\text{C}$.

To develop the technology of cooked sausage, we investigated and optimized the combined effect of hydrolyzed collagen (X_1) and cranberry powder (X_2) on five quality indicators, i. e., hardness (Y_1), cohesiveness (Y_2), color stability (Y_3), total antioxidant capacity by FRAP (Y_4), and sensory profile (Y_5). The full factorial experimental design (3×3) is described in Table 1. The factor levels were: hydrolyzed collagen (X_1) = 5, 10, 15%, cranberry powder (X_2) = 1–3%. The control sample contained no functional components. A data-driven black-box optimization strategy was adopted, and the factor-response relationships were inferred directly from the experimental data without imposing mechanistic constraints.

For each response, a second-order response-surface model was fitted in coded variables (Eq. (1)):

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (1)$$

Table 1 Experimental design and measured responses for cooked sausages

Sample	Independent variables		Dependent variables				
	Hydrolyzed collagen (X_1), %	Cranberry powder (X_2), %	Hardness (Y_1), g/mm ²	Cohesiveness (Y_2), %	Color stability (Y_3), %	Total antioxidant capacity (FRAP) (Y_4), a.u.	Sensory profile (Y_5), score
1 (Control)	0	0	3.931	85.19	87.64	38.63	4.7
2	5	1	3.865	85.44	89.50	261.91	4.5
3	5	2	3.787	83.37	91.31	547.10	4.6
4	5	3	3.686	81.06	91.60	782.34	4.3
5	10	1	3.888	83.99	88.29	267.56	4.0
6	10	2	3.746	82.02	89.62	552.75	4.1
7	10	3	3.688	79.61	90.23	787.99	3.9
8	15	1	3.847	82.54	86.60	273.22	3.7
9	15	2	3.769	80.47	88.41	558.41	3.8
10	15	3	3.668	78.16	88.70	793.65	3.5

where y is the predicted response; β_0 is the intercept; β_1, β_2 denote the coefficients of the linear terms; β_{11} and β_{22} represent the coefficients of the quadratic terms; β_{12} stand for the coefficient of the interaction term.

The statistical significance and direction of main, quadratic, and interaction effects were assessed by ANOVA and Pareto charts of standardized effects. Optimization across multiple, differently scaled criteria was performed using the Derringer–Suich composite desirability. Individual desirabilities were defined as “larger-the-better” for all of the responses, and the overall desirability was computed using the Eq. (2):

$$D = \left(\prod_{i=1}^5 d_i^{w_i} \right)^{1/\sum w_i} \quad (2)$$

where d_i is the individual desirability for the i -th response; w_i denote the corresponding importance weight.

The profiles predicted for Y_1 – Y_5 and the desirability profile were used to identify the operating region that maximizes (D) within the practical bounds $0 \leq X_1 \leq 15\%$ and $0 \leq X_2 \leq 3\%$.

The texture profile analysis (TPA) was performed on a Structurometer ST-2 texture analyzer. The samples were cut into rectangular prisms of $100 \times 20 \times 20$ mm. Each specimen was placed on the fixed lower platform and compressed by a cylindrical probe ($\varnothing 36$ mm) attached to the moving crosshead. The instrument routine “ST-2 Texture Profile Analysis TPA” was used as follows: the probe penetrated the sample to 5 mm at 0.5 mm/s, returned to the start position, and then performed a second compression to 10 mm at 0.5 mm/s before returning to the origin. The force–time data were recorded and preliminarily processed with the ST-2 software packages “ST-2” and “Algorithm”. From the TPA curves, two responses were calculated in accordance with the instrument method: hardness, g/mm², and cohesiveness, %. For each formulation, we tested three independent replicates.

The color of the cooked sausages was assessed with a CM-2300d spectrophotometer (Konica Minolta, Japan). Prior to measurements, the instrument underwent stan-

dard preparation and two-point calibration. Two disc-shaped slices for each formulation were cut from different locations. Three consecutive readings for each slice were acquired and averaged, i. e., $n = 6$ readings per formulation. Color was recorded in the CIE $L^* a^* b^*$ space: L^* (lightness), a^* (green-to-red axis; values > 0 indicate red), b^* (blue-to-yellow axis; values > 0 indicate yellow). The slices were exposed to an incandescent light source for 60 min, after which $L^*2, a^*2,$ and b^*2 were re-measured under identical settings. Color stability “S percent” was calculated as a normalized complement of the component-wise deviation between pre-exposure (L^*1, a^*1, b^*1) and post-exposure values.

The total antioxidant capacity of the samples was quantified by the FRAP method on an SF-2000 spectrophotometer (OKB Spektr, Russia). The working FRAP reagent was freshly prepared by mixing 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) dissolved in 40 mM HCl, and 20 mM FeCl₃ solution at a 10:1:1 volume ratio. Each determination combined 1.45 mL of the working reagent and 50 μ L of the sample extract (or distilled water for the blank). The mixes were incubated at 37°C in the dark for 30 min. After incubation, the suspensions were clarified by centrifugation (C-2204, Liston, Russia) at 3,600 rpm for 3 min to sediment solids, and the absorbance of the supernatants was read at 594 nm in one-centimeter path-length cuvettes. Each sample was analyzed in quadruplicate. The FRAP values were obtained from a quercetin calibration curve (140–300 μ M, Sigma-Aldrich, India; $R^2 > 0.99$) and expressed as nmol quercetin equivalents per 1 g of sample.

The sensory evaluation of the meat product followed State Standard GOST 9959-2015: *Meat and meat products. General requirements for sensory evaluation*. The analysis involved ten volunteering panelists, who were familiar with the sensory assessment criteria. The panelists provided verbal consent for the sensory evaluation. The assessment took place in a well-ventilated room with adequate lighting and no distractions. The food samples were evenly sliced and chilled to $20 \pm 2^\circ\text{C}$ to be placed in labeled white plastic plates. Each sample

was evaluated using five sensory attributes: appearance, color, smell, texture, and taste. For each attribute, the panelists assigned an independent score on a 0-to-5 point scale. For every coded sample, the mean panel score was first calculated separately for each of the five attributes across the 10 assessors. The sensory profile score (Y_5) was then obtained by averaging these five mean attribute scores, resulting in a final value ranging from 0 to 5 points.

RESULTS AND DISCUSSION

Table 2 summarizes the descriptive statistics for the five responses measured across the ten formulations of the cooked sausage system.

For every response, the location metrics showed a good agreement between the mean and the median, indicating no extreme outliers. The skewness was moderately positive for Y_2 and Y_3 and close to zero for Y_1 and Y_5 , which was consistent with distributions that are either mildly right-tailed or near-symmetric. All responses have kurtosis values close to zero, indicating that the empirical distributions have a mesokurtic (roughly normal or generalized-normal) shape.

As is common for polyphenol-driven antioxidant assays in complex food matrices, the FRAP displayed

significantly higher scatter (coefficient of variation – 54.42%) than the dispersion analysis, which displayed low relative variability for texture and color endpoints.

Table 3 was used to calibrate the desirability functions and the technological constraints, connects the empirical extreme to the corresponding factor combinations.

The practical operating range for multi-criteria optimization was displayed by combining these figures. The texture-sensory complex (Y_1, Y_2, Y_3) improved at lower X_1 (hydrolyzed collagen) and modest X_2 (cranberry powder), whereas the functional endpoints (Y_3, Y_4) improved with increasing X_2 and, for FRAP, with higher X_1 . In light of this, we set “not-less-than” thresholds for Y_2 (82%) and Y_5 (4.0) in the Derringer–Suich framework, centered a target-within-range window for Y_1 near its median, and established “larger-the-better” desirabilities for Y_3 and Y_4 .

The response Y_1 (hardness) exhibited low dispersion across the design space (standard deviation – 0.0924; coefficient of variation – 2.44%). The empirical range was 3.668–3.931, with the maximum in the control sample and the minimum in Sample 10 ($X_1 = 15\%, X_2 = 3\%$).

A quadratic response-surface model adequately described Y_1 (Eq. (3)) ($R^2 = 0.9737; R^2_{adj} = 0.9298; MS_{residual} = 0.000473$):

Table 2 Descriptive statistics of responses (Y_1 – Y_5) across the formulation space

Parameter	Hardness (Y_1), g/mm ²	Cohesiveness (Y_2), %	Color stability (Y_3), %	Total antioxidant capacity (FRAP) (Y_4), a.u.	Sensory profile (Y_5), score
Mean	3.7875	82.175	89.190	486.356	4.110
Median	3.7780	82.230	89.100	549.925	4.050
Mode	Multiple	Multiple	Multiple	Multiple	Multiple
Frequency of mode	1	1	1	1	1
Minimum	37.875	821.750	891.900	4863.560	41.100
Maximum	3.66800	78.16000	86.60000	38.63000	3.50000
Variance	3.9310	85.4400	91.6000	793.6500	4.7000
Coefficient of variation	0.01	5.72	2.49	70049.13	0.16
Standard deviation	2.43876	2.91159	1.77018	54.41857	9.82987
Standard error	0.0924	2.3926	1.5788	264.6680	0.4040
Skewness	0.02921	0.75661	0.49927	83.69536	0.12776
Kurtosis	0.146481	−0.182639	0.082608	−0.284158	0.111460

Table 3 Empirical extreme points of responses (Y_1 – Y_5) with corresponding factor settings

Empirical extreme points	Dependent variables	Values of independent variables		Response value
		Hydrolyzed collagen (X_1), %	Cranberry powder (X_2), %	
Y_1 max	Hardness, g/mm ²	0	0	3.931
Y_1 min		15	3	3.668
Y_2 max	Cohesiveness, %	5	1	85.44
Y_2 min		15	3	78.16
Y_3 max	Color stability, %	5	3	91.6
Y_3 min		15	1	86.6
Y_4 max	FRAP, a.u.	15	3	793.650
Y_4 min		0	0	38.630
Y_5 max	Sensory profile, score	0	0	4.7
Y_5 min		15	3	3.5

$$Y_1 = 3.934 + 0.006X_1 - 0.0909X_2 - 0.0003X_1^2 - 0.0006X_1X_2 + 0.0013X_2^2 \quad (3)$$

The response surface for Y_1 (hardness) was almost planar across the design space, with a clear negative gradient along the X_2 (cranberry powder) axis (Fig. 1). Hardness decreased steadily as X_2 rose, whereas the slope along X_1 (hydrolyzed collagen) was gentler, indicating a smaller softening effect. A weak curvature was visible as a slight concavity along X_1 and a marginal upward bend along X_2 , without an interior optimum. The negative interaction manifested as an extra loss of hardness when both factors were elevated simultaneously. Consequently, the highest hardness occurred at the corner with low X_1 - X_2 , and the lowest values clustered at high X_1 - X_2 , while intermediate combinations traced a smooth monotonic decline.

The Pareto chart of the standardized effects ($\alpha = 0.05$) showed that the leading term for the hardness response was the linear term of cranberry powder X_2 , $|t| = 10.474$, negative slope, implying that the hardness decreased with the increase of X_2 (Fig. 2). The linear term of hydrolyzed collagen (X_1) was less important $|t| = 1.014$, and the quadratic terms and the interaction of the two functional terms were small. The overall trend over the region of interest was thus driven by X_2 (cranberry powder), whereas X_1 (hydrolyzed collagen) had a secondary effect that became pronounced at high levels because of the negative terms.

Concerning the production process, the retention of hardness within the technological window (3.75–3.85) was most effectively accomplished by restricting X_2 (1–2%) and avoiding high levels of both factors.

The cohesiveness of the samples exhibited low dispersion across the design space (standard deviation – 2.3926; coefficient of variance – 2.91%). The observed range was 78.16–85.44, with the maximum in Sample 2 ($X_1 = 5\%$, $X_2 = 1\%$) and the minimum in Sample 10 ($X_1 = 15\%$, $X_2 = 3\%$).

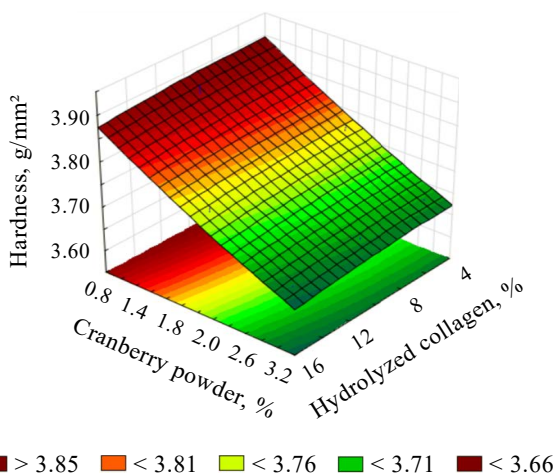


Figure 1 Response surface for hardness (Y_1) as a function of hydrolyzed collagen (X_1) and cranberry powder (X_2) in cooked sausage

A quadratic model in natural units described Y_2 (Eq. (4)) with high adequacy ($R^2 = 0.9695$; $R^2_{adj} = 0.93993$; $MS_{residual} = 0.0053398$):

$$Y_2 = 85.192 + 0.0786X_1 + 0.0046X_2 - 0.014X_1^2 - 0.031X_1X_2 - 0.439X_2^2 \quad (4)$$

The surface was concave over the region of interest: Y_2 decreased gradually with the increase of the cranberry powder (X_2), and a rise and then a decline were observed along the X_1 axis of hydrolyzed collagen (Fig. 3). The negative sign of the interaction term showed that the effect of both variables on the reduction of cohesiveness was enhanced.

The Pareto chart of the standardized effects showed that the linear effect of cranberry powder (X_2) was the most dominant effect ($|t| = 71.790$, negative), followed by the linear effect of hydrolyzed collagen (X_1) ($|t| = 48.717$) (Fig. 4). The effects of the quadratic terms and the interaction were relatively smaller.

Higher X_2 concentration increased the level of cranberry polyphenols (mainly proanthocyanidins) and organic acids, so the pH decreased and the interactions of polyphenols and proteins (hydrogen and hydrophobic bonds) with the myofibrillar proteins were enhanced.

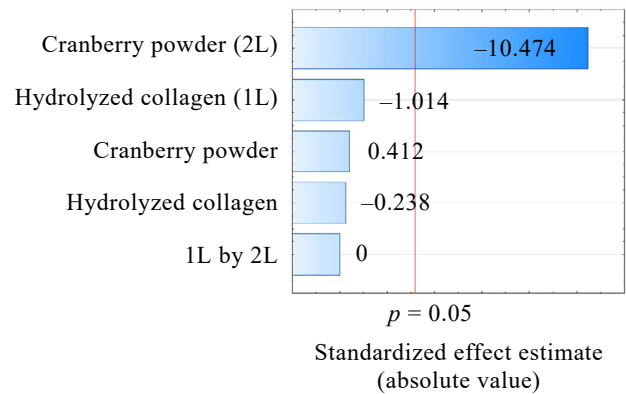


Figure 2 Pareto chart of standardized effects for hardness (Y_1)

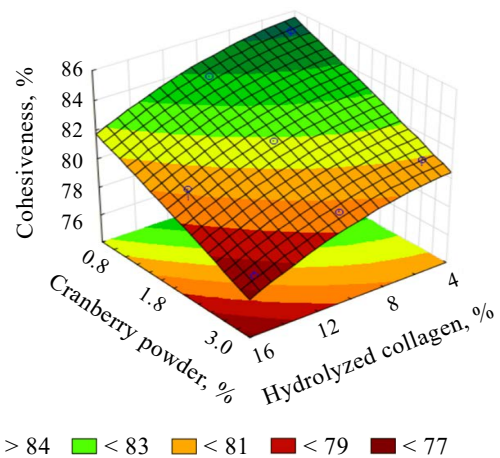


Figure 3 Response surface for cohesiveness (Y_2) as a function of hydrolyzed collagen (X_1) and cranberry powder (X_2) in cooked sausage

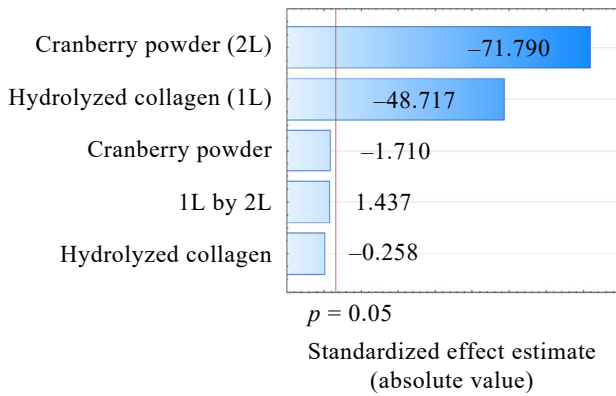


Figure 4 Pareto chart of standardized effects for cohesiveness (Y_2)

This effect decreased the extractability of the salt-soluble proteins and the protein network of the gel, so the cohesion was reduced. Collagen hydrolysate brought short peptides capable of plasticizing the gel and improving water retention. It was slightly positive in moderate levels but caused a dilution effect at the high level.

Using the framework of Derringer and Suich, the encoding of Y_2 was “not-less-than” 80%. The empirical maximum with functional variables $X_1 = 5\%$, $X_2 = 1\%$ was the evidence that cranberry powder was the key factor behind the reduction of cohesiveness.

The color stability of cooked sausages exhibited the lowest relative scatter among the responses (standard deviation = 1.5788; coefficient of variance = 1.77%). The observed range was 86.6–91.6, with the maximum for Sample 4 ($X_1 = 5\%$, $X_2 = 3\%$) and the minimum for Sample 8 ($X_1 = 15\%$, $X_2 = 1\%$). A quadratic model in natural units described Y_3 (Eq. (5)) with excellent adequacy ($R^2 = 0.99045$; $R^2_{adj} = 0.97452$; $MS_{residual} = 0.06723$):

$$Y_3 = 87.64 - 0.33X_1 + 3.9733X_2 + 0.0026X_1^2 - 0.0085X_1X_2 - 0.705X_2^2 \quad (5)$$

At low-to-moderate levels, the surface exhibited a strong positive gradient along the X_2 axis, with mild saturation at the upper end of the range (Fig. 5). The overall effect was negative but not very strong along the X_1 axis of hydrolyzed collagen. The weakly negative cross-term suggested that a high collagen content somewhat reduced the color-preserving properties of cranberry powder.

The linear term of hydrolyzed collagen (X_1) was the strongest (negative) contributor on the Pareto chart of standardized effects ($|t| = 13.933$), followed by the linear term of cranberry powder (X_2) (positive; $|t| = 10.092$) (Fig. 6). The next important term was the quadratic of Y (negative; $|t| = 3.845$). The quadratic of X_2 and the interaction were not as important. Mechanistically, the beneficial effect of X_2 aligned with cranberry phenolics (anthocyanins, proanthocyanidins) that neutralized radicals, chelated pro-oxidant iron, and inhibited the transformation of nitrosylated heme pigments into their

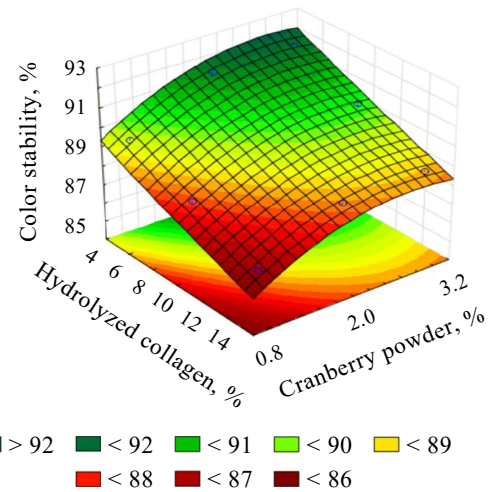


Figure 5 Response surface for color stability (Y_3) as a function of hydrolyzed collagen (X_1) and cranberry powder (X_2) in cooked sausage

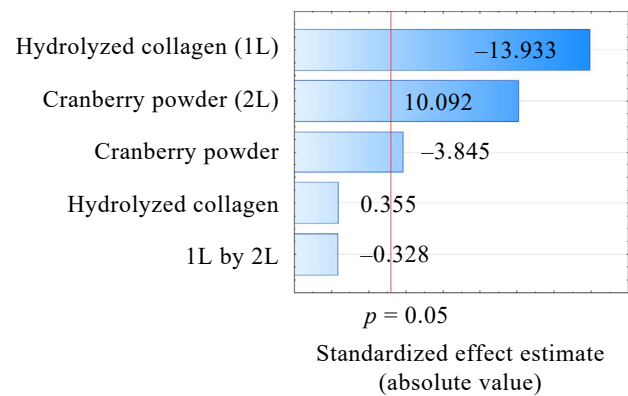


Figure 6 Pareto chart of standardized effects for color stability (Y_3)

degraded/oxidized states during storage. At the same time, the organic acids in cranberry powder lowered the pH a little, which could help stabilize the chromophores in cured meats and stop them from turning brown. The weakly negative effect of X_1 was probably due to the dilution and matrix effects. Too many collagen peptides can make water bind better and light scatter more, and they may also move or dilute pigment-bearing myofibrillar proteins.

The response surface methodology (RSM) and Pareto evidence indicated that color stability increased when X_2 approached 3% while X_1 remained around 5%, which also aligned with the texture constraints derived for Y_1 – Y_2 . This setting provided a strong color benefit with very little cost from curvature or interaction terms.

The total antioxidant capacity (FRAP) exhibited the highest relative scatter among the responses (standard deviation = 264.668; coefficient of variance = 54.42%). To stabilize variance and meet the model assumptions, we applied a logarithmic transformation to Y_4 (Eq. (6)) prior to regression. The fitted model on the log-scale showed excellent adequacy ($R^2 = 0.99772$; $R^2_{adj} = 0.99393$; $MS_{residual} = 0.00132$):

$$Y_4 = 31.6838 - 33.043X_1 + 447.59X_2 + 1.523X_1^2 + 0.889X_1X_2 - 51.73X_2^2 \quad (6)$$

The X_2 axis (cranberry powder) caused the response to rise sharply, and the negative curvature controlled a mild saturation that followed (Fig. 7). A tiny U-shape only resulted in secondary changes in relation to the X_2 axis, indicating a weak net effect along the X_1 axis (hydrolyzed collagen). The cross-term was positive and modest, indicating that collagen did not hinder and, in fact, could slightly facilitate, the antioxidant gain delivered by cranberry powder at moderate levels.

The linear effect of cranberry powder (X_2) was the primary positive contributor ($|t| = 45.45$) that outperformed all other terms, according to the Pareto chart of standardized effects (Fig. 8). The next significant effect (negative; $|t| = 6.60$) was the quadratic of cranberry powder, which indicated saturation at high amounts. Hydrolyzed collagen (X_1) had negligible effects.

Cranberry powder provided a concentrated source of redox-active polyphenols (including proanthocyanidins and anthocyanins), which directly reduced the

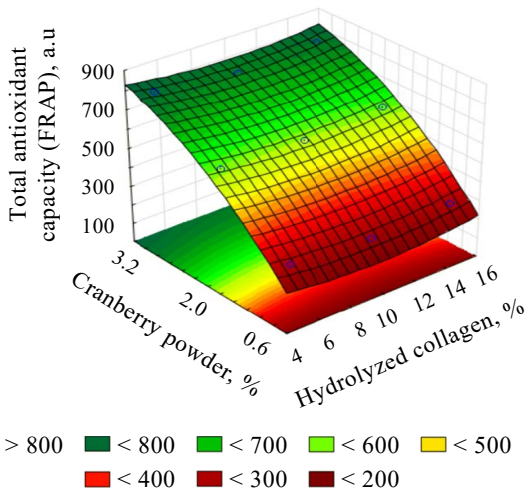


Figure 7 Response surface for total antioxidant capacity (FRAP, Y_4) as a function of hydrolyzed collagen (X_1) and cranberry powder (X_2) in cooked sausage

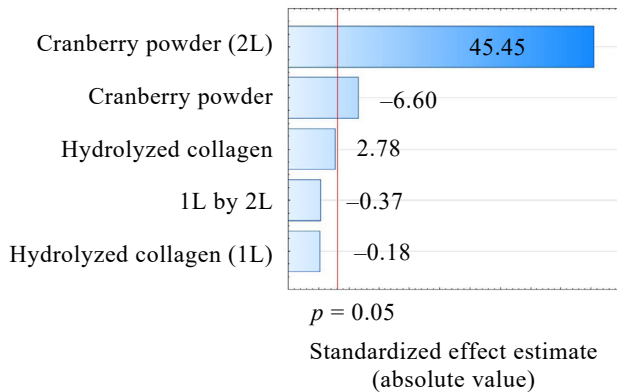


Figure 8 Pareto chart of standardized effects for FRAP (Y_4)

Fe^{3+} -TPTZ complex in the FRAP (donor-electron mechanism) assay and partially chelated transition metals, reducing prooxidant cycles. Although peptides containing donor amino acids (tyrosine and histidine) were present in hydrolyzed collagen, their contribution to reducing capacity was less significant.

To avoid going against the textural constraints (Y_1 - Y_2) of finished cooked sausages, the model and Pareto analysis made it abundantly evident that X_2 was to be maximized while X_1 was to be kept at a low-to-moderate level.

Across the design space, the sensory profile varied within 3.50–4.70, with the minimum for Sample 10 ($X_1 = 15\%$, $X_2 = 3\%$) and the maximum for the control sample. A quadratic RSM in natural units described the response with excellent fit ($R^2 = 0.99589$; $R^2_{adj} = 0.98905$; $MS_{residual} = 0.0014815$) (Eq. (7)):

$$Y_5 = 4.695 - 0.141X_1 + 0.611X_2 + 0.003X_1^2 + 0.0009X_1X_2 - 0.176X_2^2 \quad (7)$$

A slight U-shape along the X_1 axis was visible on the surface, which was far beyond the tested range (Fig. 9). As the amount of hydrolyzed collagen increased, the sensory profile gradually decreased. The response was a concave along the X_2 axis, with a distinct peak at 2%. After that, the quadratic term took over and the sensory profile declined.

These trends were confirmed by the Pareto chart: the primary (negative) driver was the linear effect of collagen ($|t| = 25.456$), followed by the quadratic ($|t| = 6.736$) and linear ($|t| = 5.303$) effects of cranberry powder (X_2) (Fig. 10). Collagen had a weaker curvature ($|t| = 2.449$). The interaction was insignificant at $\alpha = 0.05$.

Increasing the amount of hydrolyzed collagen (X_2) lowered the sensory profile score by increasing the fraction of low-molecular peptides that plasticized the protein matrix and bound water, softening the cut surface and decreasing the visual uniformity. Through pigment

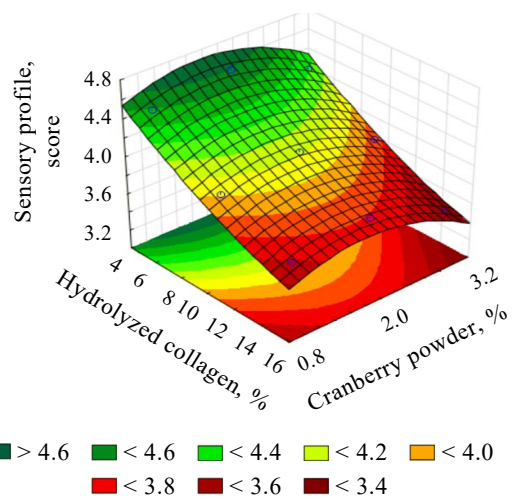


Figure 9 Response surface for sensory profile (Y_5) as a function of hydrolyzed collagen (X_1) and cranberry powder (X_2) in cooked sausage

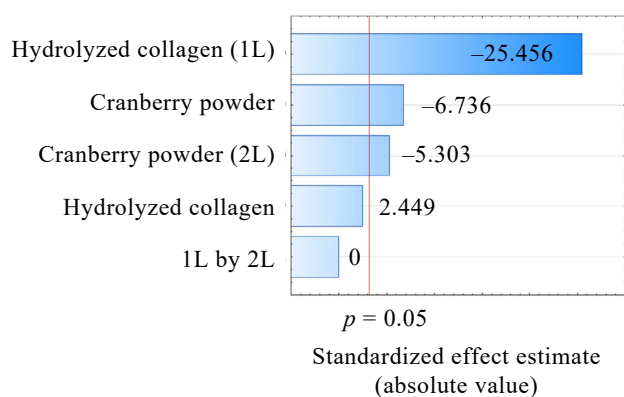


Figure 10 Pareto chart of standardized effects for sensory profile (Y_5)

Table 4 Anchor levels and desirability mapping for multi-response optimization

Dependent variable	Desirability	Level	Value of variable
Hardness (Y_1), g/mm ²	0	Low	3.668
	0.5	Medium	3.778
	1.0	High	3.888
Cohesiveness (Y_2), %	0	Low	78.16
	0.5	Medium	81.80
	1.0	High	85.44
Color stability (Y_3), %	0	Low	86.6
	0.5	Medium	89.1
	1.0	High	91.6
FRAP (Y_4), a.u.	0	Low	261.91
	0.5	Medium	527.78
	1.0	High	793.65
Sensory profile (Y_5), score	0	Low	3.50
	0.5	Medium	4.05
	1.0	High	4.60

contribution and antioxidant protection, cranberry polyphenols and anthocyanins enhanced the color vividness and surface gloss at moderate doses (positive linear term). On the other hand, too much cranberry powder caused phenolic–protein aggregation while raising acidity and astringency.

The sensory profile (Y_5) was regarded as “larger-the-better” to provide multi-response optimization. In practice, a high sensory profile was maintained while still being compatible with the texture, color stability, and FRAP targets by keeping collagen at ≤ 5 –10% and cranberry at $\leq 2\%$.

We used three anchor points, i. e., low (L), medium (M), and high (H), to set up the desirability profile to convert each response (Y_1 – Y_5) into a unitless score ranging from 0 to 1 (Table 4). These anchors, which represented the technological acceptability for cooked sausage, were extracted from the experimental domain.

The profiler used the corresponding desirability axes to overlay individual response trends against both factors (Fig. 11). The factor setting under evaluation was

indicated by the red guide lines. A balanced compromise was found when they were at $X_1 = 10\%$ and $X_2 = 2\%$, where the texture responses stayed within their acceptable bands while the color protection and antioxidant capacity were significantly enhanced. This phenomenon manifested in the desirability columns as mid-range but acceptable values for Y_1 – Y_2 and high individual desirabilities for Y_3 , Y_4 , and Y_5 , resulting in a competitive composite profile.

We contrasted the experimental measurements with the model-based predictions at the chosen formulation to confirm predictive accuracy (Table 5). All endpoints showed high levels of agreement. The absolute deviation and relative error for hardness (Y_1) were 0.035 and 0.93%, respectively. The absolute deviation of cohesiveness (Y_2) was 0.214 (0.26%). The absolute deviation for color stability (Y_3) was 0.063 (0.07%). The biggest difference was the absolute deviation in FRAP (Y_4), which equaled 7.553 (1.37%), but it was in line with the inherently higher dispersion of FRAP. The absolute deviation for the sensory profile (Y_5) was 0.017 (0.41%). The response-surface models accurately depicted the underlying behavior for formulation guidance, as all discrepancies fall within the tolerance suggested by the L-M-H anchors and within the typical analytical and process repeatability for meat systems.

The product maintained a consistent, consumer-acceptable texture and sensory qualities while achieving a strong antioxidant and color-stable profile at $X_1 = 10\%$ and $X_2 = 2\%$. This configuration maintained the collagen-derived peptides at a level that promoted water retention without softening the structure, while utilizing the redox activity of cranberry phenolics to preserve cured pigments and lipids. The desirability-driven approach was validated by the close match between predicted and observed data, which also showed that the profiler was effective in defining a narrow operating corridor around a single formulation for routine production and quality control.

Phenolic–myofibrillar interactions break down protein–protein networking and move water from protein-bound to polyphenol-associated states, loosening the emulsion gel. They can account for the softening at higher collagen-hydrolysate levels and the decrease in hardness with increasing cranberry powder levels (Table 1, Fig. 1 and 2). Stefanello *et al.* [17] found that a larger amount of cranberry pomace decreased some quality metrics, e. g., texture, which was in line with phenolics weakening the protein matrix, a vector that corresponded to our hardness trajectory. Fu *et al.* [18] also noted a decrease in hardness and chewiness as plant powder increased in pork model sausage with cherry polyphenols. They attributed this effect to the changes in protein–water interactions and oxidation control that inhibited the stiffening of cross-links. Their dose response was mirrored in our pattern at 2–3% cranberry powder.

Our cooked-sausage matrix plus berry acids produced net softening, in contrast to other studies where

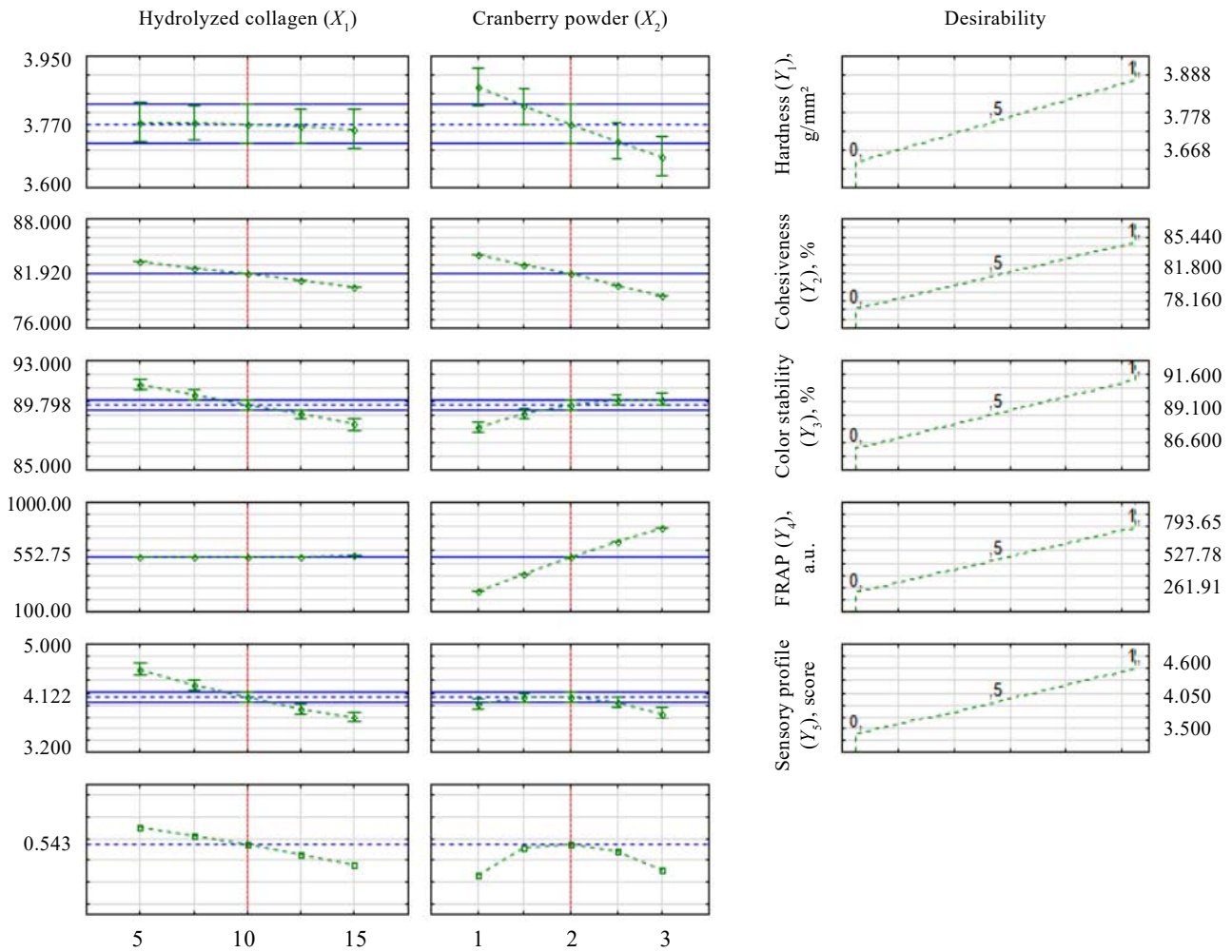


Figure 11 Overall desirability profiles of responses of cooked sausages

Table 5 Experimental and predicted response values at operating point $X_1 = 10\%$, $X_2 = 2\%$

Type of values	Hardness (Y_1), g/mm ²	Cohesiveness (Y_2), %	Color stability (Y_3), %	FRAP (Y_4), a.u.	Sensory profile (Y_5), score
Experimental	3.746	81.920	89.620	552.750	4.100
Predicted	3.781	82.134	89.557	560.303	4.117

specific flavonoids were reported to stiffen gels at specific molar ratios (rutin improving myofibrillar gel strength *in vitro*). Probably, the anthocyanin-rich, acidic cranberry system pushed the interactions beyond the strengthening window described by Jia *et al.* [19]. Additionally, Ham *et al.* [20] discovered that duck-skin gelatin hydrolysate increased cohesiveness without increasing hardness, whereas intact gelatins in some reports elevated firmness. This slight softening was observed when increasing collagen hydrolysate. It was consistent with intact gelatin vs. peptide behavior. Peptide “plasticization” might reduce hardness in our hydrolysate variations.

When the cranberry reached the upper range, cohesiveness decreased, but it increased at low-to-moderate hydrolysate (Table 1, Fig. 3 and 4). In line with the cohesiveness gains, hydroxyproline-rich peptides can provide

more interactive sites for weak protein–peptide bridging in emulsions, thus improving the internal bond recovery. Kozhakhievaya *et al.* [21] reported 1% of gelatin hydrolysate in cooked sausages. On the other hand, berry phenolics may reduce resilience on the second compression by partially blocking or rearranging myosin head interactions. Rosmawati *et al.* [22] echoed this antagonistic relationship. In their research, microstructural loosening and quality improvements occurred when cranberry powder and protease tenderization were combined in beef. This suggests that proteolysis + polyphenols can exchange cohesiveness for other quality benefits.

Our complex sausage matrix plus acidic cranberry, probably, surpassed the ideal binding ratio, which explains why our cohesiveness peaked only at peptide-rich settings. In this respect, our results were in contrast to some recent mechanistic studies which demonstrated

that certain polyphenols (catechins, resveratrol) could strengthen heat-induced gels by hydrogen bonding under controlled stoichiometry. For example, Papuc *et al.* [23] reported such concentration-dependent cross-link modulation.

Anthocyanins and polymethoxyflavonoids function as radical scavengers, metal chelators, and possible myoglobin-reducing agents. In our study, cranberry powder reduced browning and stabilized redness (Table 1, Fig. 5 and 6). Yang *et al.* [24] added 5 g/kg cranberry powder to fermented sausages, which enhanced color while forming a *Pediococcus–Staphylococcus* ecology that supported the pigment stability, a mechanism consistent with a^* retention. Karwowska & Dolatowski [25] showed that freeze-dried cranberries inhibited lipid oxidation and affected heme chemistry during storage in nitrite-free venison sausages. Their oxidation control pathway was paralleled by our color stability results.

Plant polyphenols can slow the formation of metmyoglobin at the molecular level by chelating pro-oxidant metals and donating electrons. Zhu *et al.* [26] reviewed inhibition of both lipid and myoglobin oxidation, i. e., mechanistic routes. Their results fit the higher a^* we observed in the sausage samples with cranberries. However, Liu *et al.* [27] quantified polyphenols reducing metmyoglobin and protecting pigment integrity.

Two additive chemistries, i. e., electron-donating peptides and cranberry phenolics with potent ferric-reducing power, were responsible for the noticeable increase in total antioxidant capacity (FRAP) with combined hydrolysate and cranberry (Table 1, Fig. 7 and 8). Phenolics derived from fruit were often reported to increase the antioxidant capacity in meat systems. For example, Orădan *et al.* [28] examined 18 fruits containing phenolics that improved the functional quality and oxidative stability of sausages and patties. Makangali *et al.* [29] measured significant FRAP increases in a sausage model after adding plant material (purslane). This result confirmed that botanicals incorporated into the matrix could increase the ferric-reducing capacity, which is a directionally identical effect to our cranberry-driven FRAP increase.

Cranberries have a high ferric-reducing capacity in addition to matrices. The steep FRAP gradient we observed as the cranberry dose increased were supported by Manassis *et al.* [30], who reported a notable total antioxidant capacity in cranberries. Our hydrolysate offered extra peptide-based redox buffering, which was different from single-mode antioxidants. This fact explains why FRAP in hydrolysate + cranberry variants surpassed the fruit-only benchmarks reported by other research teams.

According to the sensory profile (Table 1, Fig. 9 and 10), moderate amounts of cranberry powder enhanced the color preference and overall impression, while high amounts resulted in astringency and reduced elasticity. At intermediate amounts, the hydrolysate increased juiciness. Our dose-window was consistent with that reported by Kaldarbekova *et al.* [31], who discovered that

a high amount of powder reduced sensory acceptance while 1% powder enhanced color and texture in sausages. Uzakov *et al.* [32] described a formulation-dependent optimum rather than a monotonic benefit: higher cranberry amounts deteriorate texture and sensory profile.

Our design included only two compositional variables, so extra care must be taken when extrapolating our results to other plants. Instead of using multi-marker panels (thiobarbituric acid reactive substances, protein carbonyls), we used FRAP to infer oxidation and assessed color chemistry instrumentally, without myoglobin redox speciation. Protein–phenolic and peptide–myofibril interactions could not be microstructurally confirmed.

CONCLUSION

Based on the data obtained, the level of dispersion of the variables for texture and color was low, whereas the FRAP data varied widely (coefficient of variance = 54.42%), so a variance-stabilizing transformation was required. The hardness decreased within the design limitations with a prominent negative factor of cranberry powder and a less pronounced softening effect of hydrolyzed collagen. The quadratic model fitted well ($R^2 = 0.973$). The practical hardness range of 3.75–3.85 was ensured by adding cranberry powder at a concentration of 1–2%, avoiding the concurrent excessive use of collagen. The cohesiveness presented a concave contour and fitted well ($R^2 = 0.97$). It formed a “not-less-than” range of $\geq 82.00\%$, as well as reflected the polyphenol-induced fragilization and peptide bridging effects at the concentration of moderate collagen. The color stability ($R^2 = 0.99$) increased significantly with the addition of cranberry powder and slightly decreased with excessive collagen hydrolysate. The FRAP increased greatly with the cranberry powder, whereas collagen presented a secondary U-shaped trend of moderate intensity, enhancing from 38.63 to 793.65. The model of log-transformed data was highly adequate ($R^2 = 0.998$), distinguishing the separate redox actions of the phenolic compounds and the peptide action within a real sausage model. The sensory profile model peaked near 2.0% of cranberry.

Using the Derringer–Suich desirability functions to combine all the responses, we achieved the balanced point of hydrolyzed collagen $X_1 = 10.0\%$ and cranberry powder $X_2 = 2.0\%$. At this balanced point, color stability increased from 87.64 to 89.62%, and the total antioxidant capacity (FRAP) rose from 38.63 to 552.75 a.u. compared to the control sample. The hardness (3.931–3.746 g/mm²), cohesiveness (85.19–82.02%), and sensory score (4.7–4.1) remained within the predefined technological acceptability limits. The model confirmation at the balanced point showed close agreement between the predicted and the experimental data. The mean relative error across all responses was 0.51%, indicating that the response-surface models reliably describe the combined effect of hydrolyzed collagen and cranberry powder on the quality parameters of cooked sausages.

CONTRIBUTION

All the authors were equally involved in the manuscript and are equally responsible for any potential plagiarism.

CONFLICT OF INTEREST

The authors declared no potential conflict of interest regarding the research, authorship, and/or publication of this article.

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