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Modelling the effects of pecan nut [*Carya illinoinensis* (Wangenh.) K.Koch], roselle (*Hibiscus sabdariffa* L.), and salt on the quality characteristics of beef patties

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Abstract

The meat industry has become increasingly interested in developing products containing new ingredients oriented to consumers' health. Health benefits are gained from frequent consumption of pecan nut and roselle, which can therefore be used as ingredients in meat product formulations. However, incorporating novel ingredients or reducing the content of traditional ingredients might affect meat product quality, thus needs to be evaluated and optimised for the development of functional foods. The objective of the present work was to assess how pecan nut (0 to 10%), roselle (0 to 2%), and salt (0 to 2%) affect the physicochemical properties, antioxidant, and sensory characteristics of beef patties, and use response surface methodology to optimise the content of these ingredients in a meat product formulation. Regression models were developed to predict quality properties. All models were significant ($p < 0.05$) with an $R^2 > 0.85$ and a nonsignificant lack of fit ($p > 0.05$), thus indicating that these models could adequately predict response variables. The optimised formulation was 7.97% pecan nut, 1.59% roselle, and 1.08% salt. The predicted physicochemical properties were $L^* = 42.88$, $a^* = 12.29$, $b^* = 8.51$, pH = 5.10, and cooking loss = 24.66%; the antioxidant properties were DPPH = 0.53 mg TE/g fp, ABTS = 0.65 mg TE/g fp, and total phenolic content = 0.46 mg GA/g fp; and the sensory properties were flavour = 7.03, tenderness = 6.98, and juiciness = 7.01. Pecan nut and roselle are promising natural ingredients that can be used to prepare low-salt beef patties.

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Introduction

Modern life has facilitated changes in human activities that have resulted in increased levels of stress and sedentarism, as well as changes in traditional patterns of healthy food consumption (Chaput *et al.*, 2011). These changes have considerably affected health status, causing an increase in several diseases such as obesity, cardiovascular diseases, diabetes, and cancers (Lacombe *et al.*, 2019). The increase in the prevalence of these diseases has alarmed individuals who are concerned about their health, and who are willing to modify their present lifestyle. Health awareness motivates consumers to take care of their eating habits through the effective selection of traditional foods and to seek novel products containing ingredients that could promote health (Tuorila and Hartmann, 2020).

Beef patties are an excellent source of proteins with high biological value, essential fatty acids, vitamins, and minerals. Increased use in fast food establishments, along with convenience and low price have made beef patties one of the most popular processed meat products (Van Loo *et al*., 2020). In addition, beef patties have become a primary product for supermarkets, fast food distributors, and restaurants worldwide. However, as beef patties are a processed meat product, their consumption has been associated with cardiovascular diseases and colorectal cancer, mainly due to the saturated fat, cholesterol, and salt content of beef patties (Richi *et*

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al., 2015). The meat industry, in an effort to respond to new health-oriented markets, has been seeking bioactive ingredients that can be integrated into traditional formulations (Calderón-Oliver and López-Hernández, 2020). This direction represents a challenge for the meat industry because novel products must comply with all the quality aspects demanded by consumers. The incorporation of non-conventional ingredients into a meat formulation affects the physicochemical, sensory, and nutritional qualities of the new product. Maintaining the quality aspects of a novel product close to those of the traditional counterparts is the first step in developing alternative foods to improve nutrition. The incorporation of bioactive ingredients or the reduction of saturated fats and salt in a meat formulation are the most common strategies for developing functional meat products. Other strategies include the incorporation of fibre (Park *et al.*, 2020), natural antioxidants (Antonini *et al.*, 2020), and polyunsaturated fatty acids (Pintado and Cofrades, 2020) or the reduction of saturated fats and sodium (de Sousa *et al.*, 2020).

Pecan nut [*Carya illinoinensis* (Wangenh.) K.Koch] and roselle (*Hibiscus sabdariffa* L.) are among the ingredients having potential application in meat products. The consumption of these ingredients has been widely demonstrated in the scientific literature to provide several health benefits (Tazoho *et al.*, 2016; McKay *et al.*, 2018). Pecan nut is rich in unsaturated fatty acids, phenols, flavonoids, isoflavones, terpenes, and vitamins (Alasalvar and Bolling, 2015). Epidemiological studies show that frequent consumption of dry fruits, especially pecan nuts, is inversely correlated with cardiovascular diseases (Aune *et al.*, 2016). Reyes-Padilla *et al.* (2018) evaluated the quality of a bologna-type meat product designed for elderly individuals which incorporated pecan nut as a source of unsaturated fatty acids in combination with prunes or cranberries. The product contained high quantity of proteins, low total fat content with high monounsaturated fatty acid contents, and bioactive compounds with antioxidant effects.

Roselle is an excellent source of antioxidants, fibres, vitamins, minerals, and phytochemicals (Jabeur *et al.*, 2017). However, there have been few reports on the application of roselle in meat products. Jung and Joo (2013) optimised a combination of soybean oil and roselle extract to create a low-fat functional patty intended for industrial application. Incorporating 0.85% roselle extract into a pork patty resulted in a product with a high sensory acceptance, and suitable physicochemical properties. Similarly, Babatunde and Adewumi (2015) evaluated the quality attributes of chicken patties by adding ethanolic extracts of garlic, ginger, and roselle. The extracts provided antioxidant and antimicrobial benefits during cold storage.

Before pecan nut and roselle can be used as non-conventional ingredients in novel meat products, it is necessary to determine how these substances affect the final product quality. Response surface methodology (RSM) is a common technique used to assess the impact of using novel ingredients in meat formulations. This tool can be used to simultaneously evaluate several factors and assess linear, quadratic, and interaction effects. RSM has been used successfully in the development of novel meat products. Pérez-Báez *et al*. (2020) optimised the incorporation of roselle, potato peel flour, and beef fat into beef patties using RSM. Romero *et al*. (2019) used RSM to optimise the contents of corn starch and whey protein concentrate in a beef patty to improve the lipid profile and cooking properties. Therefore, the objectives of the present work were to evaluate the effects of pecan nut, roselle, and salt incorporation on the quality of beef patties, and to use RSM to determine the optimal combination of these components.

Materials and methods

Raw materials

Beef inside round muscle (semimembranosus) from commercial crossbred steer (3/4 *Bos taurus*) carcasses (pH 5.6 - 5.8) were purchased 48 h post-slaughter from a local market. The fresh meat (73% moisture, 5% lipids, 20% protein, and 1% ash) was transported to the laboratory under refrigerated conditions within 15 min of purchase, cut into 5 × 5 pieces, and ground in a Hobart meat grinder (Model 4152, Troy, Ohio, USA) using a 4.8-mm plate.

Roselle, pecan nut (Kirkland Signature, Costco Wholesale Corporation, Issaquah, WA, USA), and salt (Salt Bay®, Mexico) were acquired from a local market. The whole dry flower of roselle was used to preserve the active ingredients in as natural a state as possible, and eliminate further processing steps. Roselle (13% moisture, 0.3% lipids, 4.6% protein, 10.2% ash, and 71.9% carbohydrates by difference) and pecan nut (2.8% moisture, 81.8% lipids, 8.5%

protein, 1.4% ash, and 5.5% carbohydrates by difference) were ground separately in a food processor (NutriBullet NUBPT0101, Los Angeles, CA, USA), vacuum packed, and stored at -20°C until further use.

Experimental design

The effects and interactions of pecan nut (0.0, 2.03, 5.0, 7.97, and 10.0%), roselle (0.0, 0.41, 1.0, 1.59, and 2.0%), and salt (0.0, 0.41, 1.0, 1.59, and 2.0%) on the beef patty quality were evaluated using a central composite design (CCD) matrix. The maximum levels of the independent variables were determined by preliminary studies. The experimental design consisted of 20 formulations as specified in Table 1. The quality was evaluated in terms of (a) physicochemical indexes (L^*, a^*, b^*, pH, and the cooking loss), (b) the antioxidant capacity (DPPH, ABTS) and total phenolic content, and (c) sensory properties (flavour, tenderness, and juiciness). All assessments were performed on fresh patties, except for the cooking loss and sensory analyses. Two replicates of the complete experimental set were conducted, where three patties were analysed per formulation for each replicate, such that a total of six patties were used for each evaluation.

Beef patty preparation

The ground meat was divided into 20 experimental batches. Roselle, pecan nut, and salt were separately added to each batch based on the percentages specified in Table 1. Each batch was homogenised in a manual mixer (LEM Products, West Chester, OH, USA) for 3 min in a 10°C cold room. Portions weighing 70 g were formed into patties using a manual patty-forming machine. The patties were cooked following a standard procedure (AMSA, 1995) in an electric pan set at 150°C (George Foreman, Model GR2121P, Florida, USA) until the temperature in the centre of the patty reached 71°C (monitored through a thermocouple inserted into the centre of the sample).

Physicochemical properties

Instrumental colour was analysed in triplicate on the surface of the fresh patties using a Minolta spectrophotometer (Model CR-400, D65 illuminate, 10° observer angle and 11-mm aperture; Konica Minolta Sensing, Inc., Tokyo, Japan). The lightness (L^*), redness (a^*), and yellowness (b^*) were determined following CIE (Commission Internationals de I'Eclairage) colour coordinates. The pH (Hanna, Model HI 98140, and Woonsocket, RI, USA) was determined at room temperature for a 5-g uncooked patty sample homogenised in 45 mL of distilled water.

To measure the cooking loss (CL), the beef patties were cooked, cooled at room temperature (25°C), dried with a paper towel to remove excess moisture and fat, and the percentage of CL was calculated using Eq. 1:

$$\%\,\mathrm{CL} = \frac{(Raw\ weight - Cooked\ weight)}{(Raw\ weight)} \times 100 \quad \text{(Eq. 1)}$$

Antioxidant capacity and total phenolics

Extract preparation

Beef-patty extracts were prepared according to Pérez-Báez *et al.* (2020). Briefly, 10 g of each sample were homogenised with 20 mL of 80% ethanol solution (Ultra Tura® T25 basic homogenizer, IKA Works, Wilmington, NC), sonicated for 15 min (Bransonic Ultrasonic Co., Danbury, CT), and centrifuged at 10,000 *g* for 15 min at 4°C (Thermo Scientific™ Sorvall™ Legend™ X1 Thermo, MA, USA). The supernatant was collected, adjusted to a volume of 50 mL with 80% ethanol solution, and stored at -35°C until use.

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) radical scavenging assay

A DPPH assay was performed following the method reported by Palafox-Carlos *et al.* (2012) with some modifications. Briefly, an aliquot of the raw-beef-patty extract (20 µL) was mixed with 280 µL of a DPPH solution (0.0634 mM in methanol), and vortexed vigorously for 1 min. The mixture was stored in the dark at 25°C for 30 min. The absorbance was measured at 515 nm using a Fluorstar Omega UV/Visible microplate reader spectrophotometer (BMG Labtech Inc., Durham, NC, USA). A standard Trolox curve was prepared for quantification, and the results were expressed as mg of Trolox equivalent (TE) per gram of uncooked (fresh) patty (mg TE/g fp).

2,2'-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay

An ABTS assay was performed following the method reported by Re *et al.* (1999) with some modifications. Briefly, 5 mL of ABTS solution (7 mM) were mixed with 88 µL of $K_2S_2O_8$ (0.139 mM),

Table 1. Experimental combinations of treatments and experimental responses for physicochemical, antioxidant, and sensory properties of beef patties incorporated with pecan nut, roselle, and salt.

Run*	Treatment combination			Experimental response											
				Physicochemical determination					Antioxidant property			Sensory property		
	X_1	X_2	X_3	Lightness	Redness	Yellowness	pH	CL	DPPH	ABTS	TPC	Flavour	Tenderness	Juiciness
1	2.03	0.41	0.41	42.34	14.99	10.49	5.53	23.16	0.246	0.397	0.359	7.15	7.09	7.49
2	7.97	0.41	0.41	37.07	13.31	9.27	5.45	19.64	0.218	0.260	0.341	7.41	7.97	8.15
3	2.03	1.59	0.41	40.65	12.37	7.03	5.05	34.41	0.459	0.506	0.414	5.91	6.27	5.78
4	2.03	0.41	1.59	39.27	11.67	6.94	5.15	31.27	0.461	0.502	0.415	6.86	7.13	7.17
5	7.97	1.59	0.41	47.09	13.86	11.59	5.59	22.37	0.338	0.498	0.409	7.09	6.59	6.14
6	7.97	0.41	1.59	42.68	14.62	11.23	5.42	20.65	0.260	0.517	0.399	8.07	7.41	7.77
7	2.03	1.59	1.59	44.52	12.89	8.48	5.03	26.34	0.530	0.603	0.388	6.36	6.82	6.33
8	7.97	1.59	1.59	42.26	12.31	8.16	5.16	23.68	0.540	0.705	0.446	7.28	6.9	7.81
9	0	1.0	1.0	36.34	11.62	6.72	5.26	29.46	0.281	0.402	0.367	6.39	6.82	6.57
10	10.0	1.0	1.0	43.91	13.31	9.76	5.27	23.95	0.407	0.617	0.442	7.32	6.41	6.88
11	5.0	0	1.0	43.60	17.06	13.16	5.69	19.21	0.149	0.200	0.461	7.32	7.5	7.26
12	5.0	2.0	1.0	41.07	11.62	7.57	5.07	25.59	0.771	0.687	0.565	6.8	6.99	7.66
13	5.0	1.0	0	44.75	12.90	8.85	5.40	25.08	0.363	0.440	0.382	6.33	6.46	6.21
14	5.0	1.0	2.0	40.48	12.70	8.55	5.20	23.72	0.360	0.473	0.411	7.77	7.73	7.21
15	5.0	1.0	1.0	41.97	12.73	8.64	5.19	26.54	0.280	0.431	0.428	7.45	7.66	7.45
16	5.0	1.0	1.0	42.50	13.65	9.57	5.25	27.27	0.198	0.428	0.405	7.27	7.46	7.13
17	5.0	1.0	1.0	42.31	13.51	9.15	5.22	25.46	0.227	0.439	0.430	7.3	7.75	7.37
18	5.0	1.0	1.0	42.30	13.63	9.59	5.27	24.79	0.318	0.376	0.392	7.46	7.19	6.94
19	5.0	1.0	1.0	42.03	12.55	8.98	5.26	25.27	0.333	0.397	0.418	7.05	7.14	6.63
20	5.0	1.0	1.0	43.59	13.38	9.84	5.24	23.88	0.286	0.471	0.441	7.34	7.41	7.59

* Runs 1 - 8 = factorial points, 9 - 14 = axial points, and 15 - 20 = central points. X_1 = pecan nut (%); X_2 = roselle (%); X_3 = salt (%); CL = cooking loss (g/100 g meat); DPPH, mg TE/g fp = Trolox equivalent (mg/g fresh patties); ABTS, mg TE/g fp = Trolox equivalent (mg/g fresh patties); and total phenolic content, mg GAE/g fp = gallic acid equivalent (mg/g fresh patties). Sensory properties were evaluated by means of 10 cm line scale, where flavour, tenderness, and juiciness were ranked from 0 = not intense/very hard/dry to 10 = intense/very tender/moist.

and allowed to rest for 16 h in the dark at room temperature. Afterward, 1 mL of the mixture was diluted with ethanol (50%) to an absorbance of 0.7 ± 0.02 at 741 nm as the working solution. A 245-µL aliquot of the ABTS working solution was vortexed with either 5 µL of a Trolox standard solution or 5 µL of sample extract in methanol. Subsequently, the absorbance (734 nm) was measured at 1 and 6 min. The ABTS radical scavenging ability was calculated based on the Trolox standard curve, and expressed in mg of Trolox equivalent (TE) per gram of uncooked (fresh) patty (mg TE/g fp).

Total phenolic content

The total phenolic content (TPC) for the methanol-extracted samples was determined using a Folin-Ciocalteu assay (Singleton and Rossi, 1965). A 30-µL aliquot of raw-beef extract was mixed with 150 µL of Folin-Ciocalteu reagent. The mixture was allowed to stand for 5 min at 25°C, 120 µL of a Na_2CO_3 (7.5%) solution was added, and the resulting mixture was vortexed for 1 min. Afterward, the mixture was allowed to rest in the dark for 30 min at 25°C. Absorbance readings were recorded at 765 nm using a Fluorstar Omega UV/Visible microplate reader spectrophotometer (BMG Labtech Inc., Durham, NC, USA). The TPC was calculated based on a gallic acid (GA) standard curve, and expressed in milligrams of gallic acid equivalent per gram of uncooked (fresh) patty (mg GAE/g fp).

Sensory analysis

A 15-member sensory panel evaluated all the samples in separate environmentally-controlled booths (25°C and 55% RH) under red lighting. The panellists were frequent meat consumers selected from the institution's staff (8 men and 7 women aged between 25 and 38 years). Before the evaluation, two briefing sessions were conducted to acquaint the panellists with the product and terminology used. Beef patties were cooked following the aforementioned procedure, and 1.5 × 1.5 cm square pieces were cut from the centre of patties, and kept warm until tested. Twenty different formulations were evaluated over three different sessions. Each panellist randomly evaluated all the beef patties samples for sensory properties (flavour, tenderness, and juiciness) on unstructured scales of 10 cm with descriptors at either end. Each marked point was converted to a numerical value from 0 to 10 based on the location of the point. Flavour, tenderness, and juiciness were ranked from 0 = not intense/very hard/dry to 10 = intense/very tender/moist. Panellists cleansed and rinsed their palates with unsalted crackers and water between samples.

Statistical analysis

Design Expert software (V.7.6.1, Stat-Ease, Inc., Minneapolis, MN, USA) was used to determine the analysis of variance (ANOVA), determination coefficient (R^2), and lack of fit of the predictive models. The mathematical equation (Eq. 2.) corresponding to the central composite design was:

$$y = \beta_0 + \sum_{i=1}^{3} \beta_i X_1 + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i<j} \beta_{ij} X_i X_j + \varepsilon \quad \text{(Eq. 2)}$$

where, y = response variable (physicochemical, antioxidant, and sensory properties); β_0, β_i, β_{ii}, and β_{ij} = intercept, linear, quadratic, and interaction coefficients, respectively; and $X_{i\text{-}j}$ = independent variables (pecan nut, roselle, and salt contents). Models were generated using an average of six measurements of the response variables.

A numerical multi response optimisation technique (Design Expert software) was used to determine the combination of pecan nut, roselle, and salt contents that maximised the beef-patty quality. The values of the independent variables were maintained within the experimental range. The dependent variables of the instrumental colour (L^*, a^*, b^*) and pH were maintained with prescribed limits; the antioxidant capacity (DPPH and ABTS), TPC, and sensory properties (flavour, tenderness, and juiciness) were maintained at the maximum target values, and the cooking loss was maintained at the minimum target value. A batch of optimum formulations produced using RSM was prepared, and the physicochemical, antioxidant, and sensory properties were evaluated as previously described. A statistical comparative analysis of the predicted and experimental values was performed.

Results and discussion

Physicochemical properties

Table 1 shows the experimental data for the instrumental colour (L^*, a^*, b^*), pH, and CL of the beef patties. The models for the physicochemical properties are shown in Table 2. L^* ranged between 36.34 and 47.09, where the lowest and highest values were obtained for Formulations 9 (0% pecan nut, 1%

roselle, and 1% salt) and 5 (8% pecan nut, 0.4% roselle, and 0.4% salt), respectively. The lowest *a** and *b** values of 11.62 and 7.03, respectively, were obtained for Formulation 9, and the highest *a** and *b** values of 17.06 and 13.16, respectively, were obtained for Formulation 11 (5% pecan nut, 0% roselle, and 1% salt).

All the models used to estimate the instrumental colour were statistically significant ($p < 0.05$), none of the models exhibited a lack of fit ($p > 0.05$), and R^2 ranged between 0.87 and 0.97 (Table 2). These results indicated that the models were suitable for predicting the changes in the response variables to the pecan nut, roselle, and salt contents.

The colour of fresh beef patties can be altered by the type and proportion of non-conventional ingredients in the formulation (King and Whyte, 2006; Yıldız-Turp and Serdaroglu, 2010). *L** was affected ($p < 0.05$) by pecan nut, salt, and roselle incorporation, whereas *a** was only affected by roselle incorporation. The incorporation of pecan nut increased *L** and *b**, but had no effect on *a**. A similar trend was reported by Jimenez-Colmenero *et al.* (2003) for restructured beef steaks supplemented with up to 15% walnut. The increase in *L** could have been attributed to the light colour of pecan nut paste (*L** = 50.8). By contrast, roselle incorporation decreased *L**, resulting in patties with darker hues. Our results are consistent with those reported by Jung and Joo (2013) for pork patties supplemented with roselle extract (0.1 to 1.3%). Roselle incorporation had a linear negative effect and a quadratic positive effect on *a** (Table 2). This decrease in instrumental colour could have been attributed to the oxidation of the iron group of myoglobin (Salueña *et al.*, 2019) caused by the acidic nature (pH = 2.4) and high solids content of roselle. Jung and Joo (2013) reported that increasing the roselle extract concentration in pork patties caused an increase in *a** but a decrease in *b**.

The pH and CL of the beef patties ranged between 5.03 and 5.69% and 19.21 and 34.41%, respectively. The highest pH and lowest CL percentages were observed in Formulation 11 (5% pecan nut, 0% roselle, and 1% salt). The lowest pH was observed in Formulation 7 (8% pecan nut, 1.6% roselle, and 0.4% salt), and the highest CL was observed in Formulation 3 (2% pecan nut, 1.6% roselle, and 0.4% salt). Therefore, patties with lower pHs had higher CLs.

The regression models for the pH and CL were significant ($p < 0.05$), did not exhibit a lack of fit ($p > 0.05$), and yielded R^2 higher than 0.91 (Table 2); these indicators provided sufficient evidence of the suitability of the generated models for predicting these variables. The roselle content was the variable that most affected the product pH followed by the salt content, while the pecan nut content had no effect on the product pH. Roselle incorporation had a negative linear effect and a positive quadratic effect on the pH. As the roselle content in the formulation increased, the pH decreased to near the isoelectric point of the meat protein. Similar results were reported by Jung and Joo (2013) for pork patties supplemented with up to 1.3% roselle. This pH decrease was due to the acidic nature of roselle (pH = 2.4), which mainly resulted from the presence of oxalic, tartaric, and citric acids (Juhari *et al.*, 2018). It is important to consider the final product pH because of its effect on the CL (Santos *et al.*, 2016).

Roselle was the ingredient that most affected the CL of the patties (corresponding to a positive linear effect), followed by pecan nut and salt (which had a negative linear effect), and there was a negative interaction between roselle and pecan nut. The CL increased with roselle incorporation, and decreased with pecan nut incorporation. The effect of roselle incorporation could have led to the decrease in pH, whereas the effect of pecan nut incorporation could have led to the low moisture content and stabilisation of the beef patty pH. A similar trend was reported by Cofrades *et al.* (2004) for restructured beef with different walnut contents. The incorporation of walnut decreased exudate loss during heating due to a decrease in moisture and an increase in the fat content with no changes in the pH. In the present work, the increase in CL from roselle incorporation was decreased by the incorporation of pecan nut due to the negative interaction between roselle and pecan nut (Table 2). Likewise, salt incorporation decreased the CL. A similar trend in the CL was reported by Szerman *et al.* (2019) for beef patties supplemented with NaCl (0 - 2%). Salt incorporation increases the ionic strength, and causes repulsion between myofibrillar proteins, which results in the swelling of myofibrils and the partial solubilisation of filaments, thus increasing the water-holding capacity (Tornberg, 2005).

Antioxidant activity and total phenolic content

Table 1 shows the experimentally determined

Table 2. Estimated regression coefficients of models for physicochemical, antioxidant, and sensory properties of beef patties incorporated with pecan nut, roselle, and salt.

Model	Physicochemical determination					Antioxidant property			Sensory property		
	Lightness	Redness	Yellowness	pH	CL	DPPH	ABTS	TPC	Flavour	Tenderness	Juiciness
Intercept	42.44	13.24	9.29	5.24	25.51	0.27	0.42	0.42	7.31	7.43	7.00
X_1	2.19*	0.31	0.79*	0.003	-1.81*	0.04*	0.075*	0.025*	0.34*	-0.13*	0.021
X_2	-0.49*	-1.22*	-1.56*	-0.19*	2.97*	0.14*	0.11*	0.031*	-0.31*	-0.23*	-0.33*
X_3	-1.50*	-0.19	-0.18	-0.026*	-0.98*	-0.007	0.001	-0.001	0.28*	0.37*	0.53*
X_1X_2	-0.44	0.12	-0.05	-0.004	-1.98*	0.002	0.007	-0.001	0.079	0.14	0.23*
X_1X_3	-0.002	0.32	0.08	-0.007	0.29	-0.005	0.033	-0.004	0.086	-0.067	0.02
X_2X_3	0.75*	-0.04	0.15	0.06*	-0.07	0.015	0.027	-0.001	0.034	-0.057	0.035
X_1^2	-0.75*	-0.26	-0.36*	0.004	0.57	0.022	0.037*	-0.012	-0.15*	-0.28*	-0.075
X_2^2	0.03	0.40*	0.39*	0.04*	-0.95*	0.063*	0.013	0.026*	-0.079	-0.061	0.079
X_3^2	0.13	-0.15	0.20	0.02	-0.24	0.028	0.014	-0.013	-0.082	-0.11*	-0.027
P-Value	<0.0001	0.0018	<0.0001	<0.0001	0.0005	0.0002	0.0002	0.004	0.002	0.0002	0.0012
Lack of fit	0.46	0.18	0.81	0.10	0.19	0.41	0.11	0.11	0.055	0.91	0.66
R^2	0.97	0.87	0.97	0.97	0.91	0.92	0.92	0.85	0.87	0.92	0.88

* $p < 0.05$. X_1 = pecan nut; X_2 = roselle; X_3 = salt; CL = cooking loss; and TP = total phenolic content.

antioxidant capacity (AC) and TPC. Table 2 shows the regression models for the AC and TPC, and the corresponding significance, lack of fit, and R^2. The DPPH and ABTS values ranged between 0.15 and 0.77 and 0.2 to 0.7 mg TE/g fp, respectively. The lowest ACs were observed in Formulation 11 (5% pecan nut, 0% roselle, and 1% salt), and the highest ACs were observed in Formulation 12 (5% pecan nut, 2% roselle, and 1% salt) because of the high roselle content. The regression models for DPPH and ABTS were significant ($p < 0.05$), exhibited no lack of fit ($p > 0.05$), and yielded R^2 higher than 0.92.

Roselle was the non-meat ingredient that most affected the AC, followed by pecan nut, and both ingredients had positive linear effects ($p < 0.05$). Roselle incorporation increased the ACs of the beef patties. Similar findings were reported by Jamhari *et al.* (2019) in chicken sausage supplemented with roselle extract (0 - 8%). This effect on the AC is related to the content of antioxidants such as organic acids, flavonoids, and anthocyanins (Borrás-Linares *et al.*, 2015).

The incorporation of pecan nut also resulted in an increase in the antioxidant capacity, as was observed by comparing the results for Formulations 9 and 10, between which the pecan nut content was increased from 0 to 10%, while maintaining the roselle (1%) and salt (1%) contents unchanged. Pecan nut is a dried fruit containing many polyphenolic compounds with significant antioxidant capacities (Medina-Juárez *et al.*, 2018). Pecan nut has been reported to be rich in total phenolics, proanthocyanidins, gallic and ellagic acids, and flavonoids (Alasalvar and Bolling, 2015). Reyes-Padilla *et al.* (2018) evaluated the quality of a bologna-type meat product designed for the elderly that incorporated pecan nut as a source of antioxidants and unsaturated fatty acids. The antioxidant activity (DPPH) increased from 39 in the control to 60 μM TE/100 g of fresh sample in a cooked product with 5% added pecan nut.

TPC ranged from 0.34 to 0.56 mg GAE/g fp (Table 1), where the lowest value was observed in Formulation 2 (2.03% pecan nut, 0.41% roselle, and 1.59% salt), and the highest value was observed in Formulation 12 (5% pecan nut, 2% roselle, and 1% salt). The regression model was significant ($p < 0.05$), did not exhibit a lack of fit ($p > 0.05$), and yielded R^2 of 0.85; these indicators provided sufficient evidence that the obtained model was suitable for prediction of the response variable. Roselle was the component that most affected the TPC followed by pecan nut, and both components had a positive linear effect. The incorporation of roselle and pecan nut increased the TPC of the beef patties ($p < 0.05$). It has been reported in several studies that both roselle and pecan nut are rich in phenolic compounds (Fukuda *et al.*, 2003; Borrás-Linares *et al.*, 2015). Reyes-Padilla *et al.* (2018) reported an increase in TPC (μM GAE/100 g of fresh sample) from 14 in a control product to 28 in a bologna-type meat product (5% pecan nut).

The shelf life of the beef patty product incorporated with pecan nut and roselle was not evaluated in the present work. However, pecan nut and roselle are rich in antioxidant and antimicrobial compounds (Alasalvar and Bolling, 2015; Jabeur *et al.*, 2017), the incorporation of which has been demonstrated to extend the shelf life of meat products (Villasante *et al.*, 2020). Phenolics are phytochemical compounds that can inactivate and reduce the formation of free radicals in the body (Parr and Bolwell, 2000). Therefore, the incorporation of roselle and pecan nut into meat products is expected to provide nutritional and health benefits (Alasalvar and Bolling, 2015; Cid-Ortega and Guerrero-Beltrán, 2015). However, bioaccessibility and bioavailability studies need to be performed to demonstrate that roselle and pecan nut have a positive effect on human health.

Sensory analysis

Tables 1 and 2 show the experimental measurements and regression models, respectively, for the sensory properties of beef patties with added pecan nut, roselle, and salt. The sensory scores for all formulations ranged from 5.78 to 8.15, which can be considered acceptable values. All the regression models were significant ($p < 0.05$), did not exhibit a lack of fit ($p > 0.05$), and yielded $R^2 > 0.87$ (Table 2). Pecan nut was the ingredient that most affected the flavour, followed by roselle and salt ($p < 0.05$). Pecan nut and salt had a positive linear effect on the flavour, whereas roselle had a negative linear effect. The incorporation of pecan nut and salt to the beef patty formulation improved the flavour. A similar effect was observed by Jimenez-Colmenero *et al.* (2003) for a restructured meat product supplemented with 5% walnut. The incorporation of roselle to the beef patties decreased the flavour ($p < 0.05$). The negative effect of roselle on the flavour may be associated with the acidity of oxalic, tartaric, and citric acids (Juhari *et al.*, 2018).

Salt was the ingredient that most affected juiciness and tenderness, followed by roselle and pecan nut. Salt had a positive linear effect on juiciness, whereas roselle had a negative linear effect. Additionally, there was a positive interaction effect between pecan nut and roselle. That is, the effect of roselle on reducing juiciness could be improved by the incorporation of pecan nut, as shown by the positive interaction between these two ingredients (Table 2). The incorporation of pecan nut decreased the cooking loss during heating due to a decrease in moisture and an increase in the fat content, thus enhancing juiciness. Cofrades *et al.* (2004) reported that the sensory properties of restructured beef were improved by up to 10% walnut incorporation. In the present work, salt increased both juiciness and all sensory properties. Salt in meat products improves general sensory acceptance by enhancing water and fat retention, and promoting the stability of the meat matrix (Pietrasik and Gaudette, 2014).

Optimisation of the beef patty formulation

The desirability function approach is one of the most widely used methods for the optimisation of a multiple response process. In the present work, the beef patty formulation was optimised using Design Expert. The optimal beef patty formulation that maximised desirability contained 7.97% pecan nut, 1.59% roselle, and 1.08% salt. The predicted and experimental values of the physicochemical, antioxidant, and sensory properties of the optimal formulation are shown in Table 3. The experimental and predicted values were not significantly different ($p > 0.05$). Therefore, the chosen mathematical procedure adequately predicted the studied quality characteristics.

Table 3. Predicted and experimental values of physicochemical, antioxidant, and sensory properties for optimal combination* of pecan nut, roselle, and salt in beef patties.

Response variable	Predicted value	95% CI	Experimental value**
Physicochemical property			
Lightness	42.88	42.07 - 43.70	42.80 ± 1.14
Redness	12.29	11.59 - 12.99	12.26 ± 0.43
Yellowness	8.51	7.99 – 9.02	8.29 ± 0.40
pH	5.10	5.03 - 5.16	5.13 ± 0.01
Cooking loss (%)	24.66	21.88 - 26.44	23.06 ± 2.69
Antioxidant property			
DPPH (mg TE/g fp)	0.53	0.46 - 0.61	0.57 ± 0.01
ABTS (mg TE/g fp)	0.65	0.59 - 0.71	0.64 ± 0.04
Total phenolic content (mg GA/g fp)	0.46	0.42 - 0.50	0.92 ± 0.05
Sensory property* **			
Flavour	7.03	6.79 – 7.27	7.90 ± 0.50
Tenderness	6.98	6.71 – 7.26	7.06 ± 0.77
Juiciness	7.01	6.54 – 7.48	7.12 ± 1.41

* Optimal combination: 7.97% pecan nut, 1.59% roselle, 1.08% salt. ** Average ± SD of five determinations ($n = 5$). *** Sensory properties evaluated by means of a 10 cm line scale, where flavour, tenderness, and juiciness were ranked from 0 = not intense/very hard/dry to 10 = intense/very tender/moist.

Conclusion

Models were developed in the present work which could be used to predict the quality characteristics of beef patties incorporated with pecan nut up to 10%, and roselle and salt up to 2%. The optimum formulation was 7.97% pecan nut, 1.59% roselle, and 1.08% salt. The beef patty formulation was optimised using RSM to obtain a meat product with good sensory properties (score > 7). The effects of non-traditional ingredients on the beef patty quality were evaluated as a first step toward developing functional meat products. Studies on cold storage, bioaccessibility, and bioavailability of bioactive compounds are ongoing. Low-salt beef patties containing pecan nut and roselle could become an alternative food for health-conscious consumers.

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