

Effects of Roselle Extract, Potato Peel Flour and Beef Fat on the Formation of HCA of Beef Patties Studied by Response Surface Methodology [†]

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Abstract: Heterocyclic amines (HCA) are compounds formed by heating meat at high temperature. Formation of HCA in meat products should be minimized by applying different approaches. The objective of this study was to investigate the effects of roselle extract (RE, 0–1%), potato peel flour (PP, 0–2%), and beef fat (BF, 0–15%) on the formation of HCA in beef patties using response surface methodology. IQx, IQ, MeIQx, MeIQ, 4,8-DiMeIQx and PhIP were identified and quantified by HPLC. Values from 0 to 18.09 ng IQx, 0.20 to 11.66 ng IQ, 0.29 to 3.93 ng MeIQx, 0.19 to 11.51 ng MeIQ, 0.65 to 1.72 ng 4,8-DiMeIQx and 1.87 to 14.7 ng PhIP per gram of sample were obtained. All models were significant ($p < 0.05$), with no lack of fit ($p > 0.05$) and showed a determination coefficient >0.70 . Roselle extract had a negative linear effect on the formation of the total HCA, while PP and BF had positive quadratic effect. The optimal formula that minimize the formation of HCA included 0.80% RE, 0.85% PP and 7.54% BF. Roselle extract and potato peel are foods that can be used as ingredients to minimize the formation of HCA in beef patties.

Keywords: heterocyclic amines; functional meat products; roselle; potato peel; beef patties

1. Introduction

A higher incidence of cancer has been reported when the intake of fruits and vegetables is low [1], when the consumption of foods with an elevated glycemic index increases [2], or when the meat intake is high [3]. With respect to the latter one, genotoxic compounds such as heterocyclic amines (HCA) may form during cooking at high temperatures from Millard reactions between creatine, free amino acids and carbohydrates present in meat [4]. The HCA ingested through foods and subsequently absorbed into the body, are converted in genotoxic metabolites. These metabolites react with the DNA of various tissues to form adducts that derive in cancer problems.

The increase of the risk of cancer due to ingestion of HCA from food is an important issue in which is necessary to seek solutions, by reducing the formation of these compounds during meat preparation. The use of vegetable tissue or extracts, as an antioxidant source, is one of the best strategies for the reduction of HCA. This is because the polyphenolic compounds, present in some vegetables, inhibit the formation of free radicals, preventing HCA formation. The addition of calyx

or roselle extract, to meat products, has been researched as a source of natural antioxidants, however, few articles have thoroughly studied their impact on HCA formation [5]. On the other hand, potato peel contains polyphenolic compounds which in aqueous extracts have shown antioxidant activity [6]. Finally, beef fat is susceptible to oxidation which is accelerated during cooking producing free radicals that may be involved in the formation of HCA [7]. The objective of this research was to evaluate the effects of roselle extract, potato peel, and fat addition on the formation of HCA using response surface methodology.

2. Materials and Method

2.1. Experiment Design

The effects of roselle extract (RE, 0–1%), potato peel flour (PP, 0–2%) and beef fat (BF, 0–15%) addition on the formation of HCA (IQx, IQ, MeIQx, MeIQ, 4,8-DiMeIQx, PhIP) in beef patties was determined using central composite design (CCD). The experimental design consisted of 20 treatments specified in the Table 1.

Table 1. Coded and experimental values of the response variables for the CCD.

run	Coded Values			Experimental Values *			Totals HCA **
	X ₁	X ₂	X ₃	Roselle Extract	Potato Peel Flour	Fat	
1	-1	-1	-1	0.20	0.40	3.04	36.8
2	1	-1	-1	0.80	0.40	3.04	17.0
3	-1	1	-1	0.20	1.60	3.04	51.8
4	-1	-1	1	0.20	0.40	11.96	20.5
5	1	1	-1	0.80	1.60	3.04	36.4
6	1	-1	1	0.80	0.40	11.96	10.5
7	-1	1	1	0.20	1.60	11.96	45.7
8	1	1	1	0.80	1.60	11.96	11.2
9	-1.68	0	0	0	1.00	7.50	32.8
10	1.68	0	0	1.00	1.00	7.50	4.3
11	0	-1.68	0	0.50	0	7.50	26.9
12	0	1.68	0	0.50	2.00	7.50	20.5
13	0	0	-1.68	0.50	1.00	0	21.1
14	0	0	1.68	0.50	1.00	15.00	12.6
15	0	0	0	0.50	1.00	7.50	15.8
16	0	0	0	0.50	1.00	7.50	12.4
17	0	0	0	0.50	1.00	7.50	7.9
18	0	0	0	0.50	1.00	7.50	11.5
19	0	0	0	0.50	1.00	7.50	9.7
20	0	0	0	0.50	1.00	7.50	8.0

X₁: roselle extract, X₂: potato peel flour, X₃: fat, * g/100 g of product, ** ng/g., run 1–8 are factorial points, 9–14 are axial points, 15–20 are center points.

2.2. Beef Patties Preparation

All ingredients were mixed according to Table 1, and homogenized for 3 min and 70 g of the resulting batter was manually shaped into beef patties. Each beef patty was cooked for 4 min per side at 190 ± 10 °C.

2.3. Extraction of Heterocyclic Amines

The extraction of heterocyclic amines was according to Messner et al. [8] with slight modifications. One gram of lyophilized sample was homogenized in 12 mL 1 M NaOH, the suspension was shaken for 1 h at 300 rpm and room temperature. Subsequently, 13 g of diatomaceous

earth (Extrelut NT, Merck, Darmstadt, Germany) was added, homogenized and poured into a column of Extrelut NT20. Extraction was performed by washing the column with 75 mL of ethyl acetate, which was passed through an ion exchange (Waters, Milford, MA, USA) Oasis MCX cartridge (3 cm³/60 mg). The cartridge was washed with 2 mL 0.1 M HCl and 2 mL MeOH. The analytes were recovered with 2 mL ammonium hydroxide (25%) and MeOH (1/19, *v/v*) and frozen at -30 °C until further use. The samples were evaporated in a water bath at 40 °C with a constant nitrogen flow. The extracts were finally dissolved in 100 µL of MeOH for further quantification.

2.4. Chromatographic Separation

The identification and quantification of HCA followed the methodology described by Oz et al. [9] using an HPLC equipment (Agilent Technologies, 1220 Infinity LC, Waters, USA) with a diode array detector (Agilent, 1220 infinity DAD, LC, USA). The separation was carried out on a reversed phase analytical column, TSK-GEL ODS-80TM 5 µm (4.6 × 25 cm) from Tosoh Bioscience (Labkemi, Lund, Sweden) protected by a guard column (LC-18-DB, Sweden) at 35 °C. The separation was achieved with a mobile phase of methanol/acetonitrile/acetic acid/water (8:14:2:76, *v/v/v/v*) pH 5 (adjusted with 25% ammonium hydroxide) as solvent A and acetonitrile as solvent B. The flow rate was 0.7 mL/min. A gradient program (0–10 min, 0% B; 11–20 min, 0–23% B; 21–30 min, 23% B; 31–45 min, 0% B) was used. The injection volume of the final extract was 10 µL.

The identification of HCA was carried out comparing the retention time recorded by each standard and samples enriched with HCA standards at 264 nm. The recovery rate of HCA in the samples was determined by adding standards before extraction. The concentration of the HCA in the samples was calculated using a standard curve considering the indications of Haskaraca et al. [10]. Quantitative determination was performed using an external calibration curve method. A linear regression of amine concentration (versus the peak area of the amines) was performed. The regression coefficients (*R*²) for the amines were: 0.9974, 0.9958, 0.9583, 0.9852, 0.9940 and 0.9779 for IQx, IQ, MeIQx, MeIQ, 4,8-DiMeIQx and PhIP respectively.

2.5. Statistical Analysis

The response surface methodology (RSM) was used to develop the prediction models. The analysis of variance (ANOVA), the determination coefficient (*R*²), and the lack of fit of the models were determined using Design Expert (V.7.6.1, Stat-Ease, Inc., Minneapolis, MN, USA). The mathematical model corresponding to CCD is:

$$y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i < j} \beta_{ij} X_i X_j + \varepsilon$$

where *y* are the response variables (formation of heterocyclic amines), β_0 , β_i , β_{ii} , and β_{ij} are the coefficient of the intercept, linear, quadratic, and interaction respectively; and X_{i-j} are independent variables (roselle extract, potato peel flour, and fat).

The numerical optimization technique of the software Design-Expert was used for the simultaneous optimization of the multiple response. The formation of heterocyclic amines (IQ, IQx, MeIQ, MeIQx, 4,8-DiMeIQx and PhIP) was kept as minimum target.

3. Results and Discussion

Values for totals HCA are shown on Table 1. Individual HCA contents ranged from 0 to 18.09 ng IQx, 0.20 to 11.66 ng IQ, 0.29 to 3.93 ng MeIQx, 0.19 to 11.51 ng MeIQ, 0.65 to 1.72 ng 4,8-DiMeIQx and 1.87 to 14.7 ng PhIP per gram. Table 2 shows the means and regression equations obtained for the prediction of the heterocyclic amines formed during cooking of beef patties prepared with roselle extract, potato peel flour and fat. A greater effect by the incorporation of roselle extract was observed in the decrease of the studied HCA. Linear equations (*p* < 0.05) were adjusted in the formation of IQ, 4,8-DiMeIQx, and PhIP obtaining only significant decrease by the roselle extract. While the

polynomial equations of IQx, MeIQx, MeIQ, quadratic equations were obtained, observing a negative linear and positive quadratic effect by the roselle extract. A positive quadratic effect was found from the effect of the incorporation of potato peel flour, allowing the formation of MeIQx and total HCA. On the other hand, a quadratic positive effect of the added fat was observed in the formation of MeIQx.

The observed values of HCA in our beef patties are similar to some reported studies, where the effect of other vegetable extracts were evaluated [10,11]. However, it is important to highlight that the quantity of amines formed depends on the type of meat, time and type of cooking [12,13]. A significative effect of the decrease of the HCAs was found by the effect of the roselle extract, this is because the roselle is a flower rich in phenolic compounds that confers an antioxidant capacity [14]. It has been shown that antioxidants decrease the HCA formation due to antioxidants stabilizing the pirazine and/or pyrazyl radicals avoiding the union with creatinine, this way the aminoimidazole part of the amine is not formed [15]. On the other hand, the potato peel flour incorporation had a contrary effect to what was expected, increasing the MeIQx content. Potato peel flour contains a carbohydrate quantity of 275 to 1014 equivalents of glucose per gram of peel [16]. This causes an increase in glucose, which is a precursor of the HCA formation, which is causing a greater amount of MeIQx. The fat content in treatments does not have a significative impact on the HCA formation except in the MeIQx, where positive quadratic effect was found. Fat was previously reported to promote the formation of heterocyclic amines [17], however, it has been reported that a greater amount of HCA is formed in lean meat [18]. This is because, in meat with higher fat, there are fewer precursors such as amino acids and glucose for the HCA formation. Some authors report that fattier meats have greater heat transmission, so foods cook faster, thus, allowing less amines formation [19,20]. On the other hand, optimization of the ingredients used for formulation of beef patties in this study was performed as described in the materials and methods section. The desirability function approach is one of the most utilized methods for the multiple response process optimization. In this study, the optimal formulation of beef patties that minimize HCA formation was estimated using the numerical optimization tool of the Design Expert software. According to the maximum desirability of multiple responses (0.92), the optimal formulation included 0.80% of roselle extract, 0.85% of potato peel, and 7.54% of beef fat. Predicted values for the HCA formation of the optimal formulation were: 0 ng IQx, 1.89 ng IQ, 0.29 ng MeIQx, 0.04 ng MeIQ, 0.85 ng 4,8-DiMeIQx and 4.01 ng PhIP per gram of sample.

Table 2. Analysis of variance of the regression models and regression coefficients for heterocyclic amines formed during cooking of beef patties prepared with roselle extract, potato peel flour and fat.

Response	Model	Means ± SD	R ² ^a	F _{value}	Prob > F	Polynomial Equation ^b
<i>Heterocyclic amines</i>						
IQx	Quadratic	4.40 ± 3.65	0.75	3.22	0.041	0.13 - 3.74A * + 0.17B - 0.38C - 1.23AB - 0.24AC + 1.26BC + 1.62A ² * + 2.74B ² + 1.89C ²
IQ	Linear	4.32 ± 1.57	0.69	11.61	<0.001	4.32 - 2.19A * + 0.90B - 0.85C
MeIQx	Quadratic	1.74 ± 0.94	0.73	3.07	0.048	0.56 - 0.61A * - 0.28B - 0.45C + 0.37AB + 0.13AC + 0.25BC + 0.32A ² * + 0.73B ² * + 0.69C ² *
MeIQ	Quadratic	2.90 ± 1.79	0.84	5.63	0.006	1.07 - 2.65A * + 0.64B - 0.03C - 1.34AB + 0.09AC - 1.09BC + 1.37A ² * + 0.98B ² + 0.34C ²
DiMeIQ	Linear	1.14 ± 0.23	0.66	10.08	<0.001	1.14 - 0.28A * + 0.03B + 0.18C *
PhIP	Linear	6.19 ± 3.67	0.28	2.08	0.143	6.19 - 2.20A * - 0.14B - 1.14C
Totals	Quadratic	20.68 ± 6.89	0.86	6.95	0.003	10.64 - 11.67A * + 1.32B - 2.68C - 2.51AB - 1.14AC - 1.08BC + 4.50A ² * + 6.31B ² * + 3.90C ²

^a 0 < R² < 1, close to 1 means more significant. ^b A: roselle extract. B: potato peel flour, C: fat. * p < 0.05. SD = standard deviation.

4. Conclusions

The incorporation of roselle extract decreases HCA formation in beef patties, while the potato peel flour has a contrary effect. The incorporation of fat has no significant effect in the majority of the HCA, only in the MeIQx. Roselle extract and potato peel are foods that can be used as ingredients to minimize the formation of HCA in beef patties.

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