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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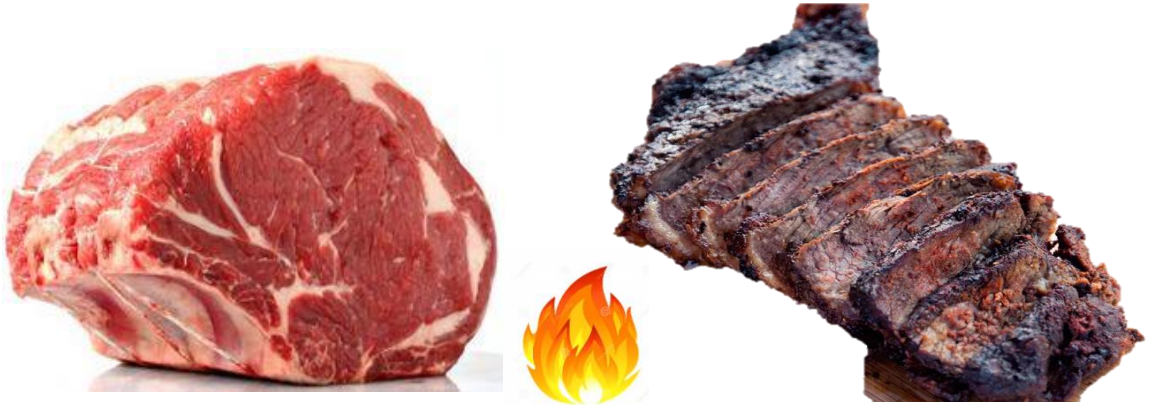
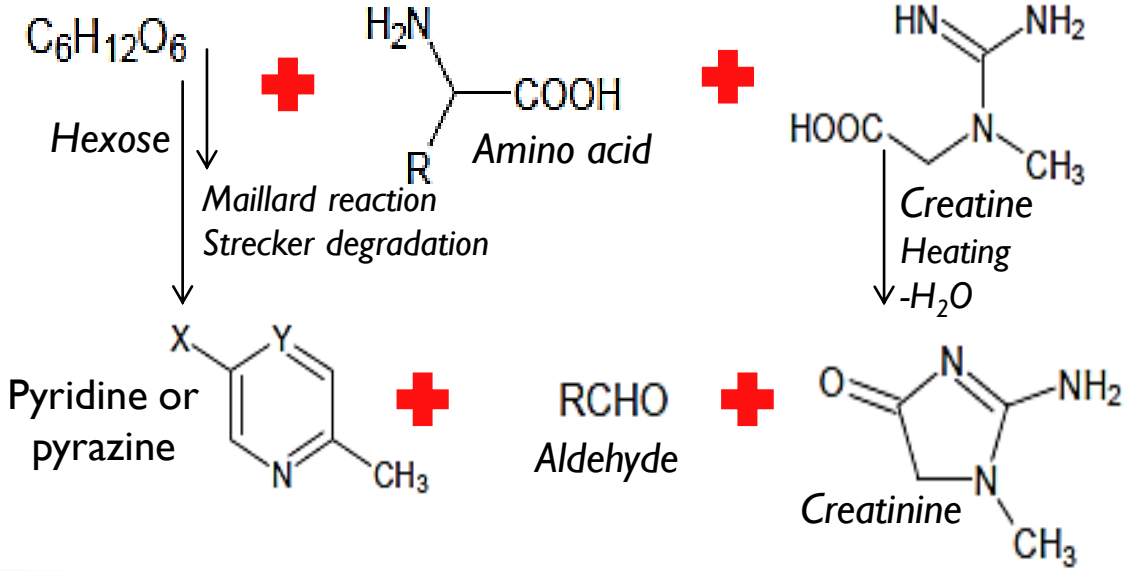
FORMATION OF HETEROCYCLIC AMINES (HCA)



170 - 200°C



Polycyclic aromatic hydrocarbons, N-nitrosamines and **Heterocyclic Amines**

initiators and promoters of carcinogenesis in humans

IQ, IQx, MeIQ, MeIQx, 4,8-DiMeIQx adn PhIP

Goldman y Shields, 2003; Linseisen et al., 2006; Jägerstad 1983; Kizil et al., 2011; Pearson et al., 1992; Rohrmann et al., 2009

MITIGATION STRATEGIES TO REDUCE THE IMPACT OF AROMATIC HETEROCYCLIC AMINES IN PROTEIN FOODS.

Antioxidants to decrease HCA

- Formation
- Absorption
- Activation

Roselle (*Hibiscus sabdariffa*)

Reducing power and trapping free radicals.
Reduction of HCA in Marinades (0.2 to 0.8g / 100g)
MeIQx 50 and 40 % reduction compared to sunflower oil

Potato peel

Aqueous extracts with antioxidants activity
Decreased the absorption of HCA at the intestinal leve
50 % MeIQx and 40 % PhIP

Meurillon y Engel 2016; Gibis y Weiss, 2010; Persson et al., 2008; Shin et al., 2002

OBJECTIVE

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.

MATERIALS AND METHODS

Preparation of roselle extract and potato peel flour

Hibiscus sabdariffa

Dry

60°C/4.5h
30°C/30min

Marinate

87°C/10min
15g/100mL

Filter and lyophilize

Solanum tuberosum

Dry

60 °C / 5 h
80 °C / 30 min
100 °C / 30 min
30 °C / 30 min

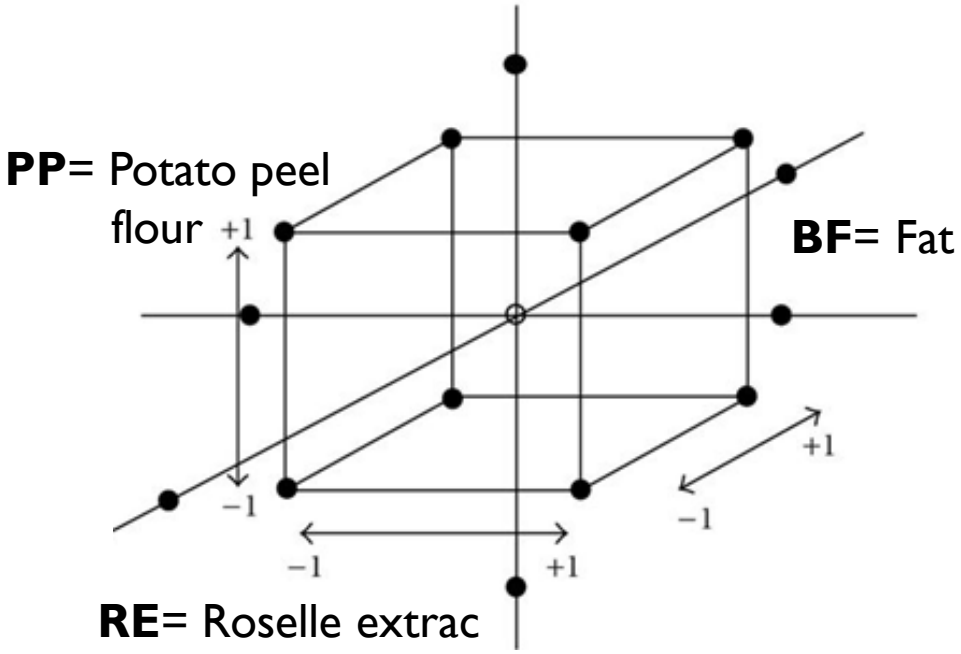
Grind and 1mm Filter

Perez-Baez *et al* 2020

Patties formulation

Response Surface Methodology

Composite core design (CCD)



Factorial 2^3

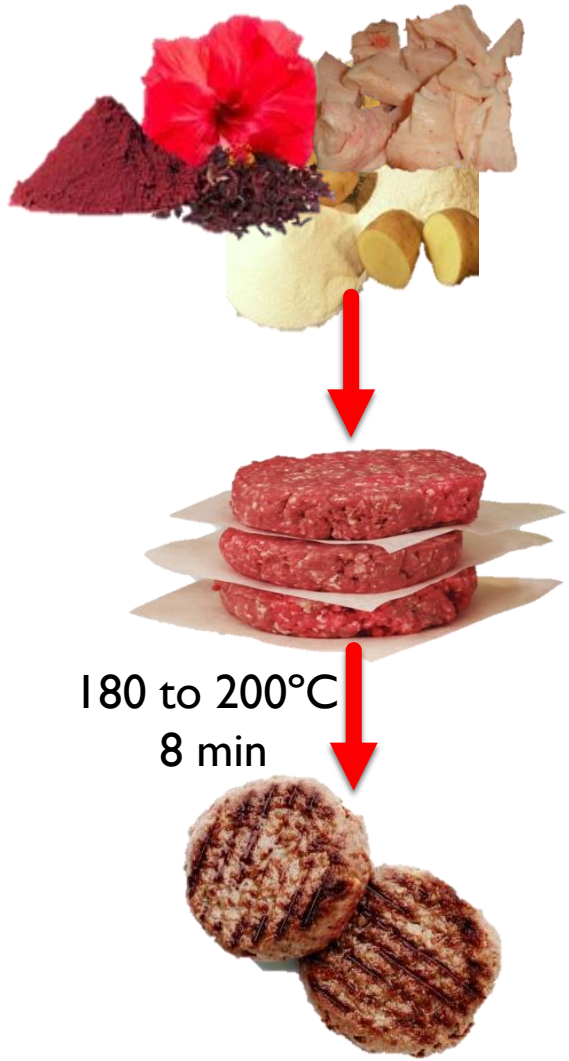
RE = 0 to 1%
PP = 0 to 2%
BF = 0 to 15%

n=3

Coded and experimental values of the response variables for the CCD

	Coded values			Experimental values		
	X_1	X_2	X_3	RE (%)	PP (%)	BF (%)
1	-1	-1	-1	0.2	0.4	3.04
2	1	-1	-1	0.8	0.4	3.04
3	-1	1	-1	0.2	1.6	3.04
4	-1	-1	1	0.2	0.4	11.9
5	1	1	-1	0.8	1.6	3.04
6	1	-1	1	0.8	0.4	11.9
7	-1	1	1	0.2	1.6	11.9
8	1	1	1	0.8	1.6	11.9
9	-α	0	0	0	1.0	7.50
10	α	0	0	1.0	1.0	7.50
11	0	-α	0	0.5	0	7.50
12	0	α	0	0.5	2.0	7.50
13	0	0	-α	0.5	1.0	0
14	0	0	α	0.5	1.0	15.0
15	0	0	0	0.5	1.0	7.50
16	0	0	0	0.5	1.0	7.50
17	0	0	0	0.5	1.0	7.50
18	0	0	0	0.5	1.0	7.50
19	0	0	0	0.5	1.0	7.50
20	0	0	0	0.5	1.0	7.50

X1: roselle extract, X2: potato peel flour, X3: fat..



HETEROCYCLIC AMINE EXTRACTION

Cooked
180-200°C
8 min
Lyophilized

1 g Sample 12 ml NaOH

13g Extrelut NT

75ml Ethyl acetate OASIS MCX Cartridge

OASIS MCX Cartridge Washing with: 2ml HCl 0.1M 2ml MeOH

The HCA are collected with 2ml of MeOH with sol. Ammonia at 25% (19/1 v / v) of the cartridges

Evaporate with nitrogen. Activation 100µl of MeOH

Gross y Grüter 1992; Haskarasca, 2014

IDENTIFICATION AND QUANTIFICATION OF HCA

High-performance liquid chromatography with a diode array detector
(HPLC-DAD)

Colum:
TSKgel ODS-80Tᴍ 5um (4.6 x 25cm)
Tosoh Bioscience

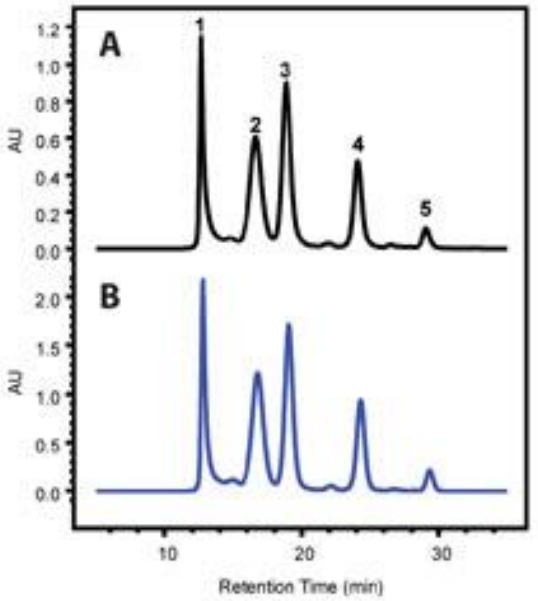
10 µL injection at 35 ° C
264nm

Sol. A
methanol / acetonitrile / water / acetic acid
8/14/76/2 v / v / v / v
Ammonium hydroxide pH 5

Sol. B
acetonitrile

Sistem agilen Technologie 1220 Infinity LC;Waters, EUA

45 min



Mattila y Kumpulainen 2002

STATISTIC ANALYSIS

DATA

Second-order complete polynomial model

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

Where:

Factors: roselle extract, potato peel flour and fat

Response variable:

AHC quantification: IQx, IQ, MeIQx, MeIQ, 4,8-DiMeIQx, PhIP and total HCA

ANOVA, R^2, Lack of fit

Design-Expert ver 11 y JMP® ver 11.0, SAS Institute

Table 1. Analysis of variance of the regression models and regression coefficients for HCA formed during the cooking of patties prepared with of roselle extract, potato peel flour and fat.

AHCs	Model	Means± SD	R^2 [a]	Fvalue	Prob > F	Linear equation[b]
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	$10.64-11.67A+1.32B-2.68C-2.51AB-1.14AC-1.08BC+4.50A^2+6.31B^2+3.90C^2$

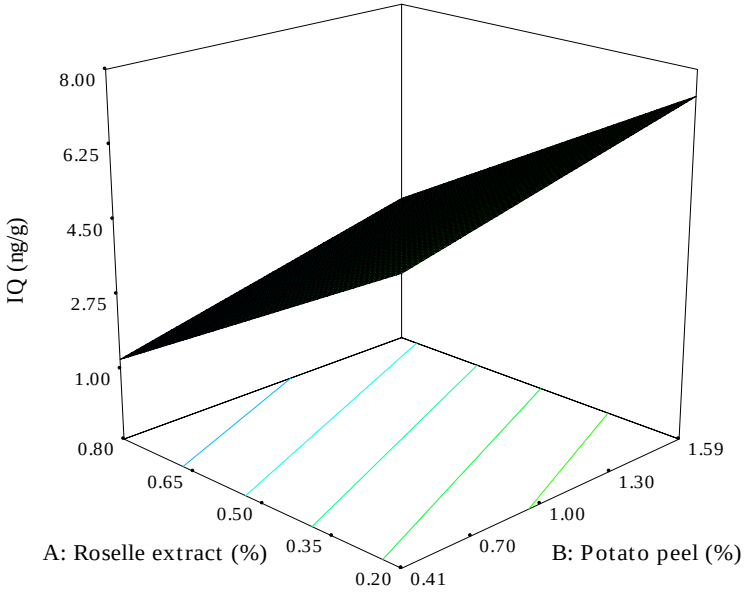
[a] $0 < R^2 < 1$, close to 1 means more significant

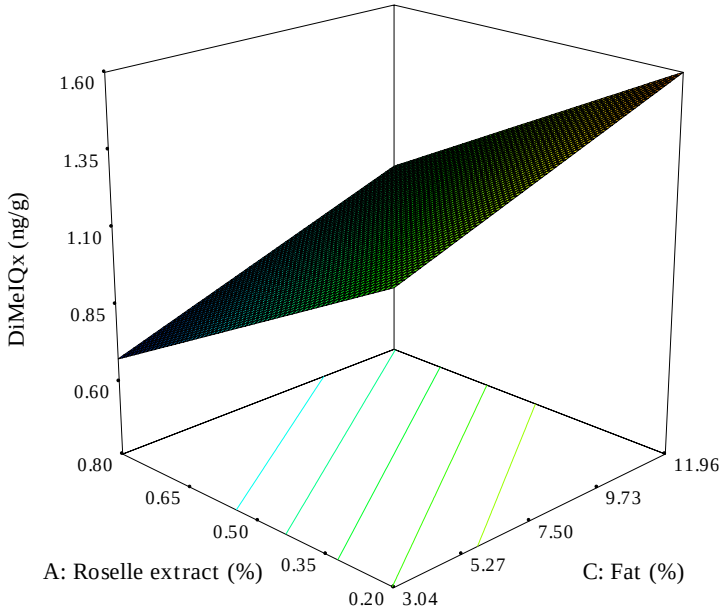
[b] A: roselle extract, B: potato peel flour, C: fat

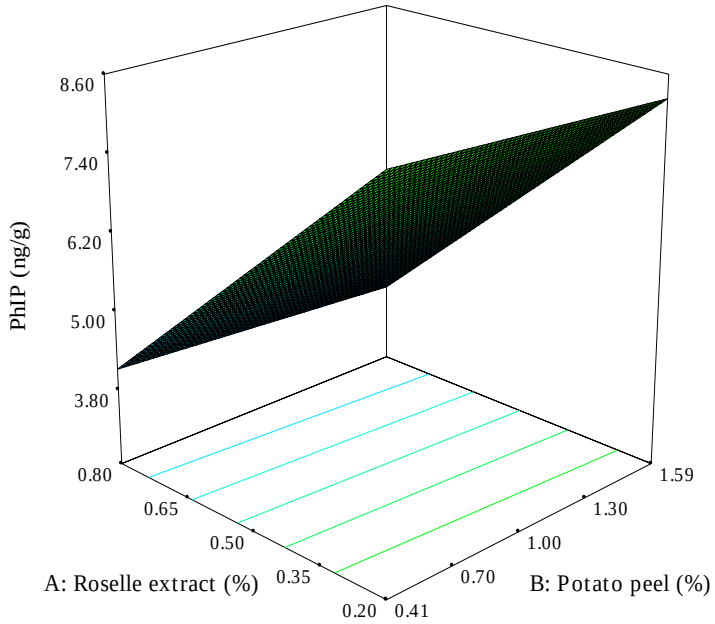
SD = standard deviation

Prediction of MeIQ, 4,8-DiMeIQx and PhIP formation in beef patties

IQ 0.20 a 11.66 ng/g patties **DiMeIQx= 0.65 a 1.72 ng/g** **PhIP= 1.87 a 14.0751 ng/g**







IQ= 4.32-**2.19A**+0.90B-0.85C

DiMeIQx= 1.14-**0.28A**+0.03B**+0.18C**

PhIP= 6.19-**2.20A**-0.14B-1.14C

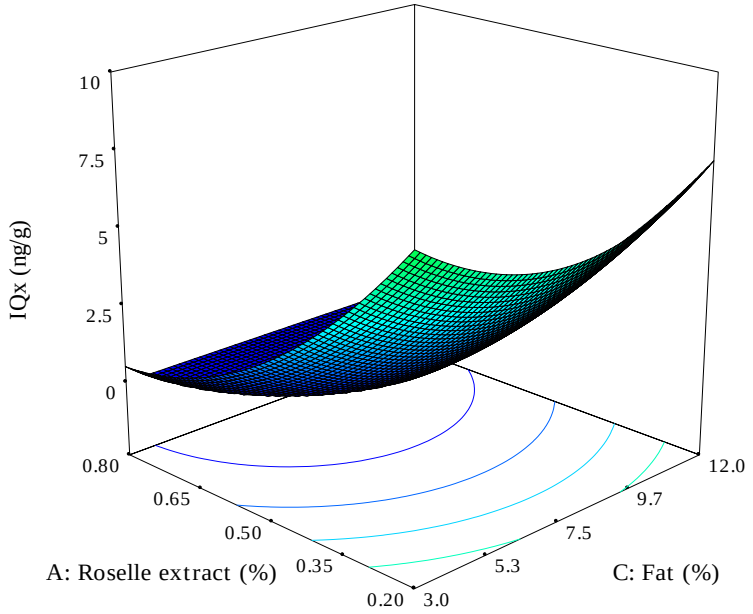
Szterk 2013
IQ 2 ng/ g
Messner y Murkovic
3, 23, 48 ng/ g
180 a 220x 10 min y 30 min

Chen et al 2017
PhIP 14.34 ± 0.36 ng/g

Gibis y Weiss 2014
PhIP 0.02–0.3 ng / g
grape seed and Rosemary Extract
90%

Prediction of IQx, IQ and MeIQx formation in beef patties

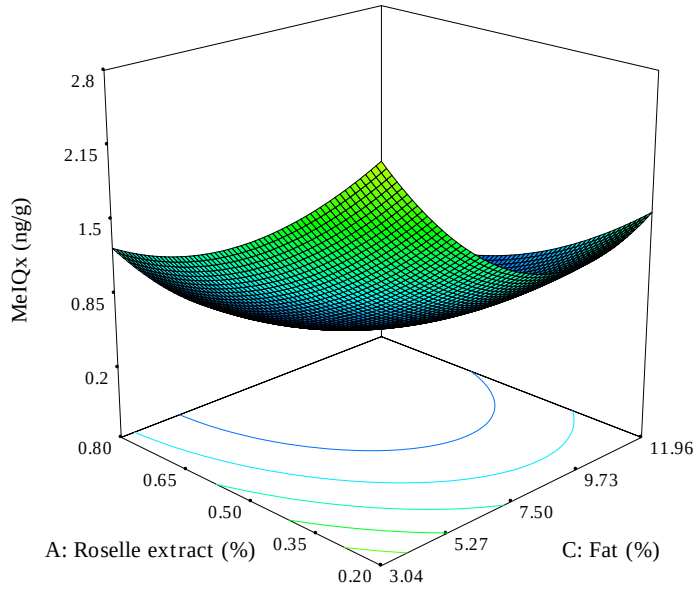
IQx 0 a 18.0939 ng/g patties



IQx= 0.13-**3.74A**+0.17B-0.38C-1.23AB0.24AC+1.26BC+1.62A^2+2.74B^2 +**1.89C^2**

Gibis y Weiss 2012
0.3–1.0 ng / g grape seed and Rosemary

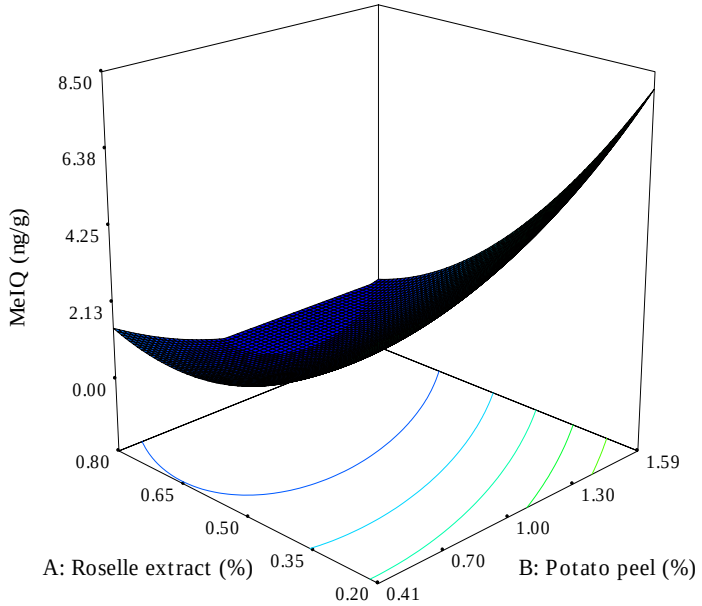
MeIQx 0.29 a 3.93752 ng/g patties



MeIQx= 0.56-**0.61A**-0.28B-0.45C+0.37AB+0.13AC+0.25BC+0 .32A^2**+0.73B^2+0.69C^2**

Chen et al 2017
MeIQx 9.94 ± 0.61 ng/g
Messner et al 2004
MeIQx 18 ng/g

MeIQ= 0.19 a 11.5136 ng/g



MeIQ= 1.07-**2.65A**+0.64B-0.03C-1.34AB+0.09AC1.09BC**+1.37A^2**+ 0.98B^2+0.34C^2

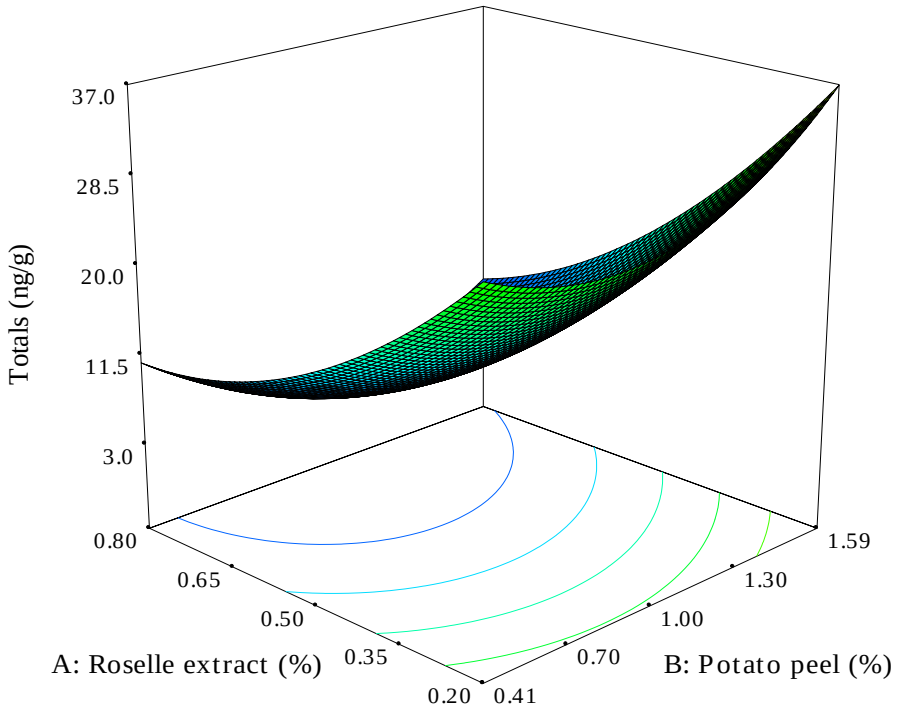
Szterk 2013
DiMeIQ 7.5 ng/ g
MeIQ 5.32 ng/g

Prediction of total amine formation in beef patties

HCA Totals= 4.34 a 51.8 ng/g patties

Sabally 2016
8.21 a 30.11ng/g total HCA

IQx e IQ



Hwang y Ngadi 2002
Lean meat 6%
Meat with 19% fat
Decrease
Gunter et al 2017
Lipid oxidation

Total de AHCs= 10.64-**11.67A**+1.32B-2.68C-2.51AB-1.14AC-1.08BC**+4.50A^2+6.31B^2**+3.90C^2

Glucosa Kiyomi Kikugawa, 1999

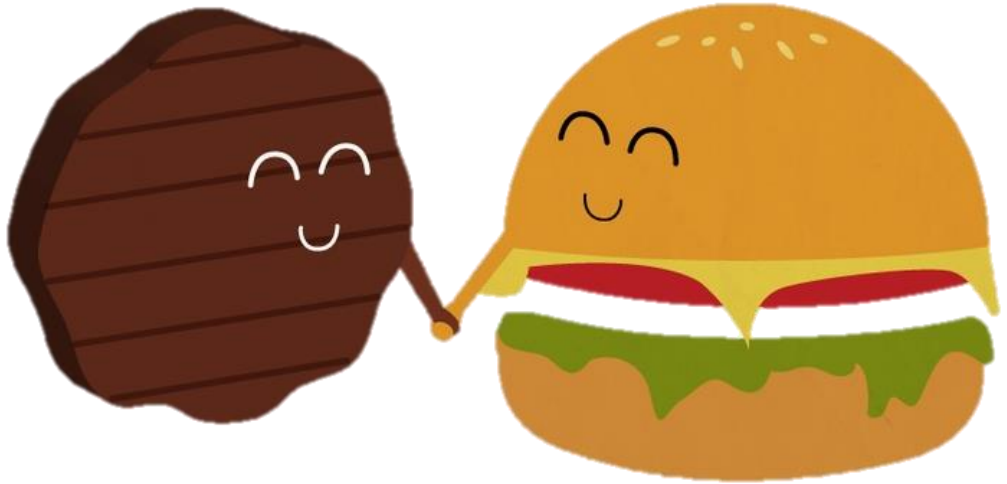
OPTIMIZATION

RE	PP	BF	Variable Response	
0.80	**0.85**	**7.54**	IQx	0 ng/g
			IQ	1.89 ng/g
			MeIQx	0.29 ng/g
			MeIQ	0.04 ng/g
			4,8-DiMeIQx	0.85 ng/g
			Totals	4.01 ng/g

CONCLUSION

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 significate 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.

Thank you!



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