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Relationship Analysis of Seven Harmful Ingredients Emission in Microwave Loose Cigarette Process Parameters and Cigarette Mainstream Smoke

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In this study, experiments and design were conducted with the method of uniform design about microwave loose cigarette process parameters. Emission of seven harmful ingredients *i.e.*, carbon monoxide, hydrogen cyanide, 4-(methylnitrosamino yl)-1-(3-pyridyl)-1-butanone (NNK), ammonia, benzo[a]pyrene (BaP), phenol and crotonaldehyde in each sample were detected. With regression analysis, genetic algorithms and other data processing methods, the data in results was processed and optimized. The results highlight the following aspects of findings: (1) Adjusting the process parameters of microwave loose and the greatest relative amplitude of emission of carbon monoxide, hydrogen cyanide, 4-(methylnitrosamino yl)-1-(3-pyridyl)-1-butanone, ammonia, benzo[a]pyrene, phenol and crotonaldehyde in the smoke of samples reached 6.64, 15.6, 15.45, 11.69, 14.06, 39.62 and 31.29 % respectively. (2) Establishing a polynomial regression equation of emission and process parameters of seven harmful components in microwave loose proves, which further enabled the realization of the integration of process parameters of low crotonaldehyde emission through the optimization of process parameters of the minimum goal, while determined significant factors of the microwave loose process affecting seven harmful ingredients.

Key Words: Cigarette, Microwave loose, Process parameters, Harmful ingredients.

INTRODUCTION

As the harmful impact of smoking on human health has been unanimously recognized and acknowledged by the international community, as promoted and organized by the World Health Organization as well as various national governments and antismoking groups, the global antismoking campaigns have been consistently improved and highlighted, leading to gradually greater pressure imposed on tobacco industries[1-3].

Carbon monoxide (CO), as one of harmful components in cigarette smoke, is the pyrolysis product of cut tobacco. Carbon monoxide is harmful to human body because it can quickly be dissolved in the human blood after it is inhaled and generate carboxyhemoglobin through the integration of hemoglobin so that the normal function of blood to carry oxygen is hindered, resulting in hypoxia and anoxemia as well as the tissue hypoxia[4,5]. Hydrogen cyanide is formed in splitting decomposition of amino acid and its related compounds of cut tobacco at 700-1000 ºC. Although accounting little in mainstream smoke, HCN has cilia toxicity, functioning as a very active inhibitor in several respiratory enzymes and imposing extremely harms on the human body[4-7]. 4-(Methylnitrosamino yl)-1-(3-pyridyl)-1-butanone is one of the specific nitrosamines in tobacco and the possible formation mechanism of 4-(methylnitrosamino yl)-1-(3-pyridyl)-1-butanone can be interpreted as: the moment nicotine is inhaled in the tobacco processing and burning, the nitrosation effect turns it into 4-(methylnitrosamino yl)-1-(3-pyridyl)-1-butanone. 4-(Methylnitrosamino yl)-1-(3-pyridyl)-1-butanone has been verified to be a strong carcinogen for all animals. Ammonia content in fresh tobacco leaves is considerably small, yet increasing with the modulated fermentation of tobacco leaves, as well as the metabolism of protein and amino acid[4,5]. Amino acid, protein, nitrate and ammonium in tobacco leaves are precursors produced by ammonia in cigarette smoke. Although appropriate amount of ammonia in cigarette is necessary for carbohydrates and cigarettes with relatively more organic acids, excessive amount of ammonia will engender intensified irritant, which not only influences the taste of cigarettes but also exert a stimulating influence on visual sense and respiratory system of human body. The constant inhalation would cause relatively serious harm on human body[4-6]. Benzo[a]pyrene is the product of incomplete combustion of tobacco at high temperature and under hypoxic conditions, which embodying high carcinogenicity[4,5]. The amount of phenol accounts for a low level in tobacco and most of the phenol products are generated in the burning process of cigarettes, exerting a strong stimulating influence on the human skin and respiratory tract membrane[4-6]. Aldehydes and ketones compounds of low molecular weight form an important class of harmful substances in cigarette smoke. Currently, eights kinds of harmful substances have been observed in the smoke. With a small amount in cigarette, most of the products are generated in the burning process. Among them, the amount of crotonaldehyde is the highest, which has intensified stimulating effect on eyes, skin and respiratory tract. Constant contact with crotonaldehyde may lead to chronic rhinitis, dermatitis, respiratory diseases and nervous system dysfunction[4-6].

Recently, more studies highlight on studying how to reduce the emission of seven harmful ingredients in mainstream smoke and reduce the hazards of smoking on human health. Kim *et al.*[8] made an attempt to add cucurbituril into cigarette filter tip so as to effectively reduce the emission of harmful ingredients such as CO in cigarette smoke. Koeckerling and Najmann[9] proposed to add an oxidant into cigarette filter tip at room temperature so as to completely remove CO from cigarette smoke. Velichko *et al.*[10] processed raw materials containing polysaccharide with the mixture of phosphoric acid, dimethylformamide and urea and added the product into cigarette filter tip through the drying and grinding process so as to adsorb CO and other substances in cigarette smoke. Callaway *et al.*[11] invented a cigarette smoke filter with resin of surface area to quality ratio constituted by chitosan derivative so as to selectively filter HCN in cigarette smoke. Maillard and Hadad[12] invented a special filtering system, which can selectively filter benzo[a]pyrene and nitrosamine and other harmful ingredients while exerting no influence on the amount of nicotine and fragrance in cigarette smoke. Waddel *et al.*[13] have found that spraying alcoholic solution on cut tobacco can cut off the selective location of nitrosamine in tissues. Yoshida *et al.*[14] processed cigarettes with iodic acid alkali metals brine solution, which can disintegrate tobacco specific nitrosamine in cigarettes and reduce its amount. Ya *et al.*[15] applied four types of porphyrins and extracts of phenolic compound plants to produce additives in cigarettes, which can significantly reduce benzo[a]pyrene and 4-(methylnitrosamino yl)-1-(3-pyridyl)-1-butanone in cigarette smoke. According to researches of Han *et al.*[16], filtering efficiency of ammonia and acetate filter in cigarette smoke is much better than modified C-fiber filter tip. Wen *et al.*[17] proposed to add natural extracts of dihydro-myricetin from ampelopsis grossedentata into cigarettes so as to reduce the amount of crotonaldehyde and phenol in cigarette smoke. Nie *et al.*[18] have developed a binary filter rod to reduce the amount of crotonaldehyde in cigarette smoke.

Tobacco processing is an important part of cigarette production as well as the key to determine the quality of cigarettes, which also exerts influences on the changes of chemical compositions of cigarettes[19]. Yet, no report has focused on the impact of emissions of seven harmful ingredients in mainstream smoke from processing parameters. This paper, based on cigarette production process, selected the microwave loose process to analyze the relationship between seven harmful ingredients in mainstream cigarette smoke and process parameters.

EXPERIMENTAL

C-type cured tobacco leaf group formula (made by Fuling Branch Factory of China Tobacco Chuanyu Industrial Corporation).

Sodium hydroxide (AR grade), potassium cyanide (AR grade), Chloramine T (AR grade), potassium hydrogen phthalate (AR grade), isonicotinic acid (AR grade), 1,3-methyl barbituric acid (AR grade), 37 % concentrated hydrochloric acid (AR grade), Brij35 solution (poly ethoxy lauryl ether, AR grade), dichloromethane (HPLC grade), anhydrous sodium sulfate (AR grade), basic alumina (AR grade 200-300), *N*-pentyl-(3-methyl pyridyl) nitrosamine (standard), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (standard), methyl sulfonate, conductivity water, methanol (HPLC grade), cyclohexane (HPLC grade), benzo[a]pyrene (standard), 9-phenyl anthracene (standard), deionized water (0.45 μm membrane filter), acetonitrile (HPLC grade), acetic acid (AR grade), phenol (standard), tetrahydrofuran (HPLC grade), isopropanol (HPLC grade), perchloric acid (AR grade), pyridine (AR grade), 2,4-dinitrophenylhydrazine (AR grade), crotonaldehyde 2,4-dinitrophenyl hydrazone derivative compounds (standard).

Devices: Tunnel-leave conditioning machine (Kunming Shipbuilding Co. Ltd.); SH315D tube plate cut tobacco drying machine (Qinhuangdao Tobacco Machinery Co. Ltd.); RM20/CS smoking machine (Germany Borgwaldt company); AB204-S analysis balance (Switzerland Mettler-Toledo Company); HY-8 speed oscillator (Guohua Electric Appliance Co. Ltd.); Skakar flow analyzer (Netherlands Skalar company); gas chromatography-thermal analysis spectrometer (PE-CSI company); ICS-3000 ion chromatograph (Dionex corporation USA); HP6890GC/5973MSD (U.S. Agilent company); DELTA D200H ultrasonic generator (Taiwan Delta Company); V-805/R-205 vacuum rotary evaporator (Switzerland Buchi corporation); HP1100 liquid chromatograph (USA Agilent company).

Experiment design method

Experiment design: Uniform design is developed by Chinese mathematician Wang Fangtai and Wang Yuan to integrate number theory with multivariate statistics and it is a new design method applying to multi-factor and multi-level experiences on the basis of the orthogonal design. Compared with the orthogonal design, uniform design performs traits such as less experiment times and good uniform dispersion and so on[20].

The method adopts DPS statistical software (DPS ® v12.01 data processing system) and identifies factors[21], levels and adjustment programs for process parameters based on the factors and level selection principle of uniform design as well as the actual production conditions (Table-1).

TABLE-1
MICROWAVE LOOSE PROCESS PARAMETER DESIGN TABLE

Test number	Microwave power (KW)	Process time (s)
WB–01	180	300
WB–02	150	360
WB–03	120	240
KB–2	140	320

Adjustment and sampling method of processing parameters: According to the pilot program, the process parameters of microwave loose were adjusted. When the drying process was operating steadily and smoothly, according to the processing time of the materials in the process, three times (about 25 Kg/time) of sampling were conducted at equivalent time intervals about 1.5 meter from the exit of the cut tobacco drying machine. After the sampling, it was required to spread the tobacco leaves immediately on the clean surface until it was naturally cooled to room temperature. And then the leaves were mixed uniformly. With the application of inquartation, a sample of about 40 Kg was reserved, cited as WB-01 and WB-02 and WB-03 were obtained based on the same method. The drying leave samples underwent the natural balance of water to 11.8 %-12.5 % and then all samples were packed by the same machine platform. The raw materials, assistance materials, weight, circumference, length and other parameters of cigarettes under production were in strict consistence with the process standards of brand cigarettes. The control sample KB-2 was the production under normal standards of process and production process before the parameter adjustment.

Detection methods

Carbon monoxide: The detection shall be conducted according to "YC/T 30-1996 Cigarette-determination of carbon monoxide in the gas phase of smoke-NDIR method".

Hydrogen cyanide: The detection shall be conducted according to "YC/T 253-2008 Cigarette-determination of hydrogen cyanide in cigarette mainstream smoke-continuous flow method".

4-(Methylnitrosamino yl)-1-(3-pyridyl)-1-butanone: The detection shall be conducted according to "GB/T 23228-2008 Cigarette-determination of tobacco specific *N*-nitrosamines in total particulate matter of mainstream Cigarette smoke-GC-TEA method".

Ammonia

Sample preparation: In accordance with the provisions of GB/T5606.1, cigarettes were selected as laboratory samples. The end of cigarette smoke trap was connected with collecting trap. According to the provisions in GB/T19609, these samples were smoked. Twenty pieces of cigarettes were smoked in each round. 50 mL absorption solution ((0.01 mol /L) hydrochloric acid, similarly herein after) was added into the collecting trap. After the smoking process, the filter with particulate components and one-fourth of the filter of the clean collecting trap was put in the extraction flask (250 mL). Precisely 50 mL absorption solution was then added. The extraction flask was then placed on the oscillator for the oscillation and extraction of 40 min, so as to obtain the extraction of mainstream particulate smoke phase. 5 mL of the mainstream smoke particulate absorption liquid in the collecting trap gas and the above mentioned extraction liquid was quantitatively removed and blended in a 50 mL polypropylene plastic flask. Constant volume was achieved by absorption liquid. After shaking uniformly, with the 0.45 μm water phase membrane, the solution was filtered and the product was placed in a chromatography flask, as testing sample solution.

Device conditions: The detection was achieved by ion chromatography. Ion chromatographic conditions: mobile phase A: 0.05 mol/L methanesulfonic acid; mobile phase B: water; mobile phase gradient conditions were listed in Table- 2. Flow rate: 1.2 mL/min; injection volume: 25 μL; column temperature: 30 ºC; suppressor current: 90 mA.

TABLE-2
LEACHEATE GRADIENT ELUTION PROCESS

Time (min)	A (%)	B (%)
0.0	32	68
6.0	32	68
6.1	50	50
14.5	50	50
14.6	32	68
28.0	32	68

Calculation of ammonia content: Calculation was based on the retention time qualitative, peak area and the standard curve method (concentration of working standard solution is shown in Table-3).

TABLE-3
SERIES WORKING STANDARD SOLUTION (μg/mL)

Series working standard solution	1 #	2 #	3 #	4 #	5 #
NH_4^+	0.1	0.3	0.5	0.7	0.9

For each sample, the parallel testing was conducted twice and the ammonia content in the mainstream smoke can be calculated in accordance with the following function:

$$M = (A \times V \times a \times 17)/(n \times 18)$$

where, M-ammonia content in the samples (μg/cig); A-determined concentration of ion chromatography in the samples (μg/mL); V-constant volume of samples (mL); a-the quality dilution magnification of device under test, which is 10 by the rotary smoking machine; n-the number of cigarettes smoked (sticks).

Take the arithmetic mean of two parallel samples for the test results and the result is accurate to 0.01 μg/cig. The relative average deviation between the results of parallel determination is no more than 10 %.

Benzo[a]pyrene: The detection shall be conducted according to "GB/T 21130-2007 Cigarette-determination of benzo[a]pyrene in total particulate matter in the total particulate matter".

Phenol: The detection shall be conducted according to "YC/T 255-2008 Cigarette-determination of major phenolic compounds in mainstream cigarette smoke-high performance liquid chromatography method".

Crotonaldehyde: The detection shall be conducted according to "YC/T 254-2008 Cigarette-determination of major carbonyls in mainstream cigarette smoke-high performance liquid chromatography method".

RESULTS AND DISCUSSION

Intuitive analysis: According to the test program given in experimental section, process parameters of microwave loose was adjusted and the test samples were under detection on aspect of emissions of phenol, 4-(methylnitrosamino yl)-1-(3-pyridyl)-1-butanone, CO, HCN, ammonia, benzo[a]pyrene and crotonaldehyde in cigarette mainstream smoke. The results were shown in Figs. 1-7. Figs. 1-7 can demonstrate that among the mainstream smoke, the content of WB-01 was the lowest as 15.21 ng/cig; 4-(methylnitrosaminoyl)-1-(3-pyridyl)-1-butanone content was the lowest with WB-03 as 3.94 ng/cig; CO, HCN, ammonia, benzo[a]pyrene, crotonaldehyde emissions were lowest with KB-2, respectively as 14.34 mg/cig, 116.00 µg/cig, 13.98 µg/cig, 12.10 ng/cig and 10.85 µg/cig. Among test samples, phenol, benzo[a]pyrene and HCN emissions in mainstream smoke in WB-02 were the highest, respectively as 25.19 µg/cig, 14.08 ng/cig and 137.40 µg/cig; 4-(methyl-nitrosamino yl)-1-(3-pyridyl)-1-butanone content was found highest with KB-2, as 4.66 ng/cig; CO content was found highest with WB-03 as 15.36 mg/cig; ammonia and crotonaldehyde content was found highest with WB-01, respectively as 15.83 µg/cig and 15.79 µg/cig. By the adjustment of the process parameters of microwave loose samples, maximum relative amplitude of 4-(methylnitrosamino yl)-1-(3-pyridyl)-1-butanone, CO, HCN, ammonia, benzopyrene and croton aldehyde emission reached 39.62, 5.45, 6.64, 15.6, 11.69, 14.06 and 31.29 %, respectively.

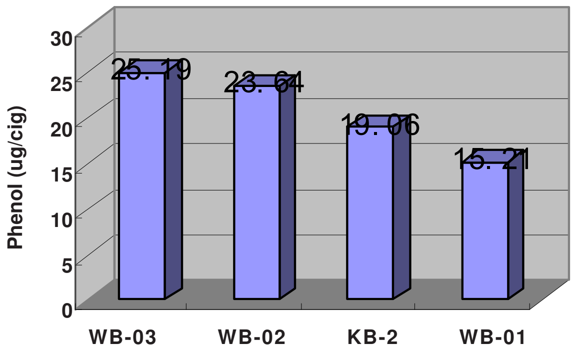

Fig. 1. Sample phenol content variation diagram of microwave loose process

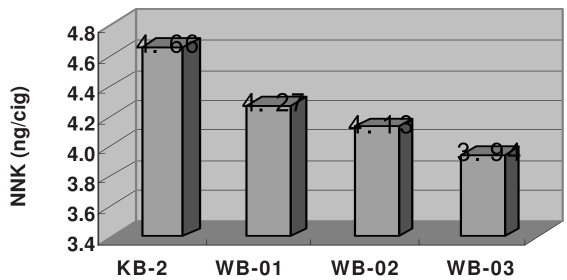

Fig. 2. Sample 4-(methylnitrosamino yl)-1-(3-pyridyl)-1-butanone content variation diagram of microwave loose process

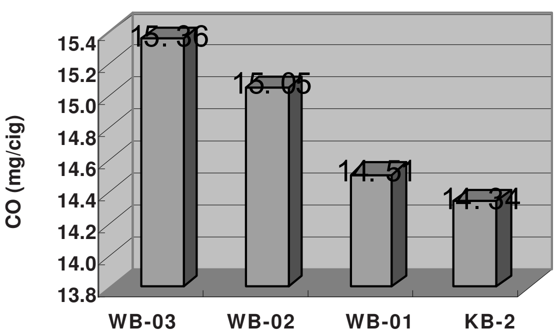

Fig. 3. Sample CO content variation diagram of microwave loose process

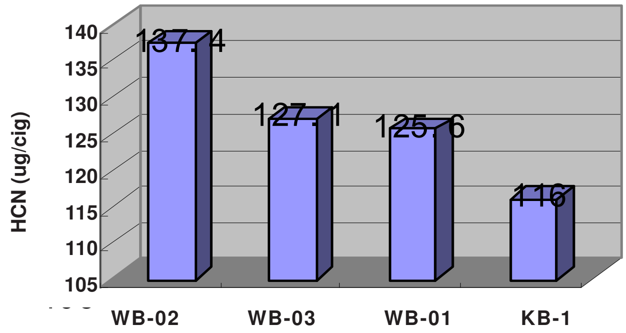

Fig. 4. Intuitive analysis diagram of sample HCN content of microwave loose process

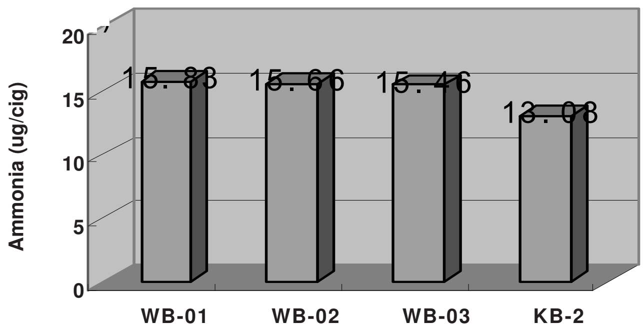

Fig. 5. Intuitive analysis diagram of sample ammonia content of microwave loose process

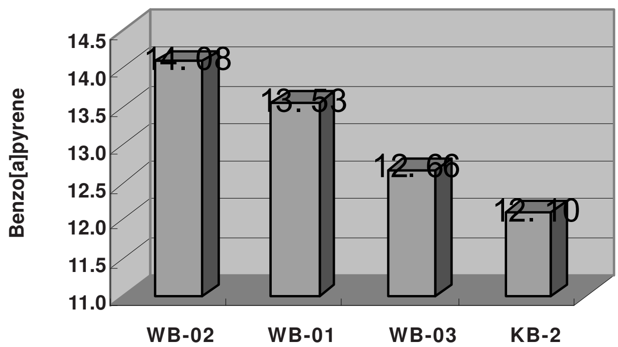

Fig. 6. Intuitive analysis diagram of sample benzopyrene content of microwave loose process

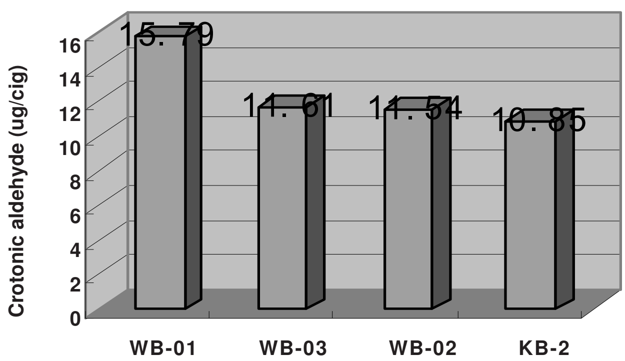

Fig. 7. Intuitive analysis diagram of sample crotonic aldehyde content of microwave loose process

TABLE-4
REGRESSION EQUATION OF SEVEN HARMFUL INGREDIENTS AND MICROWAVE LOOSE PROCESS PARAMETERS AS WELL AS EQUATION TEST FINDINGS

Index	Regression equation	Significant level	Adjusting coefficient of determination
Benzo[a]pyrene	$y = 10.9469 + 0.00004x_1x_2$	0.3638	0.1071
Phenol	$y = 30.2649 - 0.0004x^2_1$	0.2871	0.2622
HCN	$y = 401.3633 - 1.9447x_2 + 0.0033x^2_2$	0.5839	0.0000
Crotonaldehyde	$y = 57.4980 - 0.686x_1 + 0.0025x_1^2$	0.0439	0.9942
CO	$y = 16.4473 - 0.0110x_1$	0.4150	0.0134
NH_3	$y = 14.2353 + 0.00004x_1^2$	0.6000	0.0000
NNK	$y = -6.3934 + 0.0701x_2 - 0.0001x_2^2$	0.5348	0.1418

X_1 indicates microwave power (KW) and X_2 indicates process time (s); NNK = 4-(Methylnitrosamino yl)-1-(3-pyridyl)-1-butanone.

Experiment optimization of the relationship between process parameters and emissions of seven harmful ingredients

Regression modeling and fitting and the significance of the equation test: Based on uniform test results, the polynomial regression equations for emissions of seven harmful ingredients of the microwave loose process and process parameters were established respectively, along with the test of the fitting and significance of the established equations. The results were shown in Table-4. It can be seen from Table-4 that the significance level was larger than 0.05, indicating that regression equation built on the level of 0.05 showed no significance with no obvious linear relationship between independent variables and dependent variables in the equation. The significant level of the regression equation based on crotonaldehyde and microwave loose process parameters was smaller than 0.05, indicating that regression equation built on the level of 0.05 showed significance with obvious linear relationship between independent variables and crotonaldehyde. When crotonaldehyde has a linear relationship with the process parameters, the adjusting coefficient of determination of the equation was larger than 0.99, indicating that the fitting data of the regression equation of crotonaldehyde and process parameters is good with relatively greater goodness of fit in the equation. The independent variables in the regression equation holds a significant and effective interpretation role of crotonaldehyde.

Model diagnosis: The aforementioned polynomial regression equation over 0.05 levels was made into standardized residual-predictive value chart and statistics were collected from the established model so as to test of the linear relationship, the standard deviation, the existence of distinguished value and to determine whether the model can be accepted. The diagnosis results were shown in Fig. 8. Standard residual-predictive value chart in Fig. 8 indicates that the distribution of standard residuals of crotonaldehyde is between ± 2.0, indicating that there is no distinguished value; the scatter diagram indicates no obvious trend, demonstrating that the established linear regression equation was eligible; the distribution of the standard residuals in scatter diagram was in the long stripe area ± 1.5, indicating that the equation meets the requirements of mean-square deviation. The test results of the linear relationship, the standard deviation, the existence of distinguished value showed that the regression equation of crotonaldehyde and microwave loose process parameters can be accepted for optimization analysis.

Factor analysis: Table-5 shows the impact of dependent variables from independent variables in the regression equation of microwave loose process. It can be seen from Table-5 that the significant level of the impact was practically less than 0.5, indicating that the interaction effect of microwave power and process time on benzo[a]pyrene, that of microwave power on phenol, crotonaldehyde, NH_3 and CO and the effect of processing time on HCN and 4-(methylnitrosamino yl)-1-(3-pyridyl)-1-butanone was not significant.

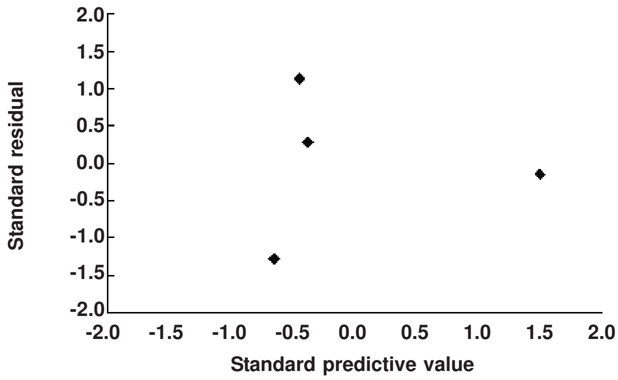

Fig. 8. Residual crotonaldehyde-predictive value map

TABLE-5
IMPACT OF DEPENDENT VARIABLES FROM INDEPENDENT VARIABLES IN THE REGRESSION EQUATION OF MICROWAVE LOOSE PROCESS

Index	Variables	Direct coefficient	Significant level
Benzo[a]pyrene	x_1x_2	0.6362	0.3638
HCN	x_2	-11.0998	0.4302
	x^2_2	11.4346	0.4210
CO	x_1	-0.5850	0.4150
NNK	x_2	11.5032	0.3925
	x^2_2	-11.0986	0.4033
Phenol	x^2_1	-0.7129	0.2871
Crotonaldehyde	x_1	-7.5916	0.0567
	x^2_1	8.4454	0.0510
NH_3	x^2_1	0.4000	0.6000

Note: X_1 indicates microwave power (KW) and X_2 indicates process time (s); NNK = 4-(Methylnitrosamino yl)-1-(3-pyridyl)-1-butanone.

Optimization of test parameters: For accepted models, the genetic algorithm was used in the optimization of process parameters for minimum desired value so as to get the process parameter combination of low desired value of each process (Table-6). Table-6 shows that the optimization of the established and accepted equation with the genetic algorithm can produce the process parameter combination of low crotonaldehyde content: microwave power 135 KW, process time: 320 s.

TABLE-6
PROCESS PARAMETER COMBINATION OF LOW DESIRED VALUE OF MICROWAVE LOOSE PROCESS

Index	Optimized parameter combination	
	Microwave power (KW)	Process time (s)
Crotonaldehyde	135	320

Validation of optimization results: Cigarettes of a certain brand were selected and validation test was conducted for the obtained microwave loose process parameter combination of low emission of crotonaldehyde through the contrast with cigarettes produced under normal process of cigarette production and processing standards. The results are shown in Table-7.

TABLE-7
CROTONALDEHYDE TESTING RESULT OF ORIGINAL SAMPLE AND OPTIMIZED SAMPLE

Name	Microwave power (KW)	Process time (s)	Crotonaldehyde emission (μg/cig)
Original sample	140	320	12.89
Optimized sample	135	320	10.94

As can be seen from Table-7, the application of optimized microwave loose process parameter combination in the production can effectively reduce the emission of crotonaldehyde in the mainstream smoke by 22.88 %.

Conclusion

The impact of emissions of benzo[a]pyrene, phenol, HCN, carbon monoxide, ammonia and 4-(methylnitrosaminoyl)-1-(3-pyridyl)-1-butanone from microwave loose process parameters can not reach a significant level. But it exerts significant influence on the emission of crotonaldehyde. The establishment of effects model with relatively high precision has been achieved.

The optimization of low desired value by testing process parameters can produce the combinations of process parameters of low emissions of crotonaldehyde. The validation results have demonstrated that the application of the combinations of process parameters of low emissions of crotonaldehyde can effectively reduce the emission of crotonaldehyde by 22.88 %.

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