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Development of Methodology for Detection of Formaldehyde-DNPH in Milk Manager by Central Composite Rotational Design and GC/MS

Desenvolvimento de Metodologia para Detecção de Formaldeído-DNPH no Leite por Design Rotacional da Composição Central e CG/EM

Desarrollo de Metodologia para Detección de Formaldehído-DNPH en Lechera por Central Composite Rotational Design y GC/MS

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Everton Barbosa Vaz
ORCID: https://orcid.org/0000-0002-7735-6350
Universidade Federal do Espírito Santo, Brazil
E-mail: evertonbvaz@hotmail.com
Mario Ferreira Conceição Santos
ORCID: https://orcid.org/0000-0003-1981-834X
Universidade Federal do Espírito Santo, Brazil
E-mail: mariosantos408@gmail.com
Ester Gonçalves de Jesus
ORCID: https://orcid.org/0000-0003-4091-2138
Universidade Federal do Espírito Santo, Brazil
E-mail: estergonacalves551@gmail.com
Karla Moreira Vieira
ORCID: https://orcid.org/0000-0001-6755-7135
Universidade Federal de Ouro Preto, Brazil
E-mail: karlavieira@ufop.edu.brorci
Vanessa Moreira Osório
ORCID: https://orcid.org/0000-0001-5156-4568
Universidade Federal do Espírito Santo, Brazil
E-mail: moreirava@yahoo.com.br
Luciano Menini
ORCID: https://orcid.org/0000-0003-3656-5428
Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo, Brazil
E-mail: imenini@ifes.edu.br

Abstract
Milk is among the food more consumed by humanity. In this way, a study of its physical-chemical characteristics is justified to analyze if its components are by the legislation and if its hygiene conditions, more precisely the use of formaldehyde as a preservative, are established. The International Agency for Research on Cancer warns that formaldehyde has physicochemical properties that make it carcinogenic when consumed in food. In Brazil, the legislation does not establish minimum values for the presence of formaldehyde in foods of animal origin, and the technique suggested by the Ministry of Agriculture, Livestock, and Supply is a qualitative test. With the large consumption of milk by the Brazilian population and because it is a food subject to adulteration. The present work sought to analyze milk in terms of its physical-chemical characteristics (fat, density, freezing point, non-greasy solids, lactose, and proteins) and develop a methodology to analyze formaldehyde derivatized with 2,4-dinitrophenylhydrazine in milk samples using gas chromatography coupled to mass spectrometer.
Keywords: Milk; Formaldehyde; Physicochemical; Chromatography; Derivatization; 2,4-Dinitrophenylhydrazine.

Resumo
O leite está entre os alimentos mais consumidos pela humanidade. Dessa forma, justifica-se um estudo de suas características físico-químicas para analisar se seus componentes estão de acordo com a legislação e se suas condições de higiene, mais precisamente o uso de formaldeído como conservante. A Agência Internacional de Pesquisa sobre o Câncer alerta que o formaldeído possui propriedades físico-químicas que o tornam cancerígeno quando consumido em alimentos. No Brasil, a legislação não estabelece valores mínimos para a presença de formaldeído em alimentos de origem animal, e a técnica sugerida pelo Ministério da Agricultura, Pecuária e Abastecimento é um teste qualitativo. Com o grande consumo de leite pela população brasileira e por ser um alimento sujeito a adulteração; o presente trabalho buscou analisar o leite quanto às suas características físico-químicas (gordura, densidade, ponto de congelamento, sólidos não gordurosos, lactose e proteínas) e desenvolver uma metodologia para analisar formaldeído derivatizado com 2,4-dinitrofenilhidrazina em amostras de leite, usando cromatografia gasosa acoplada a espectrômetro de massa.
Palavras-chave: Leite; Formaldeído; Físico-químico; Cromatografia; Derivatização; 2,4-Dinitrofenilhidrazina.

Resumen
La leche es uno de los alimentos más consumidos por la humanidad. De esta forma, se justifica un estudio de sus características físico-químicas para analizar si sus componentes están a la altura de la legislación y si están establecidas sus condiciones de higiene, más precisamente el uso de formaldehído como conservante. La Agencia Internacional para la Investigación del Cáncer advierte que el formaldehído tiene propiedades fisicoquímicas que lo hacen cancerígeno cuando se consume en los alimentos. En Brasil, la legislación no establece valores mínimos para la presencia de formaldehído en alimentos de origen animal, y la técnica sugerida por el Ministerio de Agricultura, Ganadería y Abastecimiento es una prueba cualitativa. Con el gran consumo de leche por parte de la población brasileña y por ser un alimento sujeto a adulteración. El presente trabajo buscó analizar la leche en cuanto a sus características físico-químicas (grasa, densidad, punto de congelación, sólidos no grasos, lactosa y proteínas) y desarrollar una metodología para analizar formaldehído derivatizado con 2,4-dinitrofenilhidrazina en muestras de leche, mediante cromatografía de gases acoplada a espectrómetro de masas.
Palabras clave: Leche; Formaldehído; Fisicoquímico; Cromatografía; Derivatización; 2,4-Dinitrofenilhidrazina.

1. Introduction

Milk is a product from the complete milking of healthy and well-fed cows. It must be refrigerated and kept at a temperature of 7°C on the rural property, reaching the industry with a maximum of 10°C (BRASIL, 2018). It is the primary raw material for manufacturing numerous products, from dairy products such as dairy drinks, curds, and cheeses, to pasteurized milk and ultra-high-temperature milk (UHT milk). (Alves, 2008).

UHT milk is milk that has been heated for 2 seconds at 130°C in a continuous flow and immediately cooled to a temperature below 32°C (Brasil, 1997). According to Tronco (2008), the UHT process aims to eliminate the vegetative forms of microorganisms present in milk. The milk quality that will reach the industry will depend on its composition parameters, physical-chemical characteristics, and raw milk hygiene (Hayes and Boor, 2001; Silva et al.; 2009). For example, a product with added water or some preservative substance can be considered adulterated or falsified (Brasil, 1997).

According to data from the International Agency for Research on Cancer, formaldehyde is among the 25 most-produced chemical substances worldwide, and its production exceeds 21 million tons annually. (Seow et al., 2015; Anema et al., 2019) mentions that the IARC classified it as carcinogenic, tumorogenic, and teratogenic because it produces anomalies in humans. However, some experimental studies showed that formaldehyde is harmful to some animal species. Therefore, using this aldehyde in the food industry seeks to delay or inhibit the growth of bacteria that can harm foods.

There are numerous procedures for extracting and analyzing formaldehyde from different samples. However, almost no procedures use Gas Chromatography coupled with mass spectrometry (GC-MS). According to De Freitas Rezende et al. (2017), analytical techniques such as HPLC and GC-MS are the ones that show better results because they are more selective and sensitive for the determination of formaldehyde not only in milk but in beers, fish (Chen et al., 2008 and 2009), water (Hill et al., 2009) and juices (Ruiz-Jiménez & De Castro, 2006). However, as it is a very volatile and consequently unstable compound, formaldehyde must undergo a derivatization reaction to being analyzed by HPLC and GC-MS. The derivatization reaction makes it more stable, selective, and easier to detect.

Currently, in Brazil, the method used to detect formaldehyde in milk samples consists of a qualitative method of minor sensitivity where the presence of formaldehyde in raw milk and UHT no established values. (Nascimento et al., 2017). However, the European Food Safety Authority establishes that daily exposure to formaldehyde from food of animal origin should not exceed 100 mg/kg of food per day (EFSA, 2014). Thus, this article aimed to analyze physicochemical parameters and antibiotics present in milk. In addition, optimize a methodology through Central Composite Rotational Design (CCRD) for extracting formaldehyde in milk samples using derivatization with 2,4-Dinitrophenylhydrazine for GC/MS analysis.

CCRD has the main objective of finding operating conditions that maximize the system's response (Shenbaga et al., 2015). The main feature of the CCRD is the optimization of the extraction process, improving the combinations of variables in the process.

2. Experimental

2.1 Materials

The equipment used in this work was a Shimadzu Gas Chromatograph coupled to a Shimadzu Mass Spectrometer (GCMS_QP2010 PLUS). Master Mini Analyzer (17HS8402), QUIMIS microprocessor pH meter, Baby I Centrifuge (206). to derivatization of formaldehyde was used the 2,4-Dnitriphenylhydrazine Derivatizing Reagent (Dynamic).

2.2 Sample

Twelve different samples of UHT milk brands were collected, each with two different batches in the shops in the cities of Alegre/ES, Itaperuna/RJ, and Viçosa/MG, identifying the samples as to their brand and lot as A1, A2, B1, B2, C1, C2, D1, D2, E1, E2, F1, F2, G1, G2, H1, H2, I1, I2, J1, J2, K1, K2, L1, L2.

2.3 Physicochemical analysis

The analyses determined fat, density, freezing point (cryoscopic index), protein, lactose, and non-fat solids (defatted dry extract). The Master Mini Milk Analyzer was used to analyze all samples. Thus, 20 mL of milk sample was taken and placed in the mini master analyzer, and after around 30s obtained, the result.

2.4 Antibiotic analysis

The presence of Beta-Lactams and Tetracyclines were analyzed in the milk samples. It used an ECO BT rapid test were detects the antibiotics. A volume of 200 µL of the milk sample was measured and added to the container containing the ECO BT test reagent. After 5 min, we obtained positive or negative results.

2.5 Derivatization with 2,4-Dinitrophenylhydrazine

An excess solution of 0.0375g of DNPH was prepared and dissolved in 500.00 mL of acetonitrile. The solution was used in the extraction optimization and for the milk samples' derivatization. The extraction and derivatization were performed according with a section 2.7 (Vogel et al., 2000).

2.6 Chromatographic conditions

The chromatographic conditions used were: a temperature ramp starting at 190°C and being increased by 10°C per minute until reaching a temperature of 260°C, remaining for 1 min and increasing again by 30°C per min until reaching the final temperature of 290°C. The injector temperature was 250°C, the temperature of the ion source in the mass spectrometer was 250°C, the interface temperature was 200°C, the solvent cut-off time was 3 min, and the carrier gas was the Helium. The formaldehyde retention time was 4.95 min, and the total run time was 9 min.

2.7 Development of methodology for detection of formaldehyde

A standard solution of 10 ppm formaldehyde in acetonitrile/DNPH was analyzed to obtain the retention time and ions (m/z) characteristic of the analyte of interest. The injections were accordingly to the chromatographic conditions described in the previous item.

2.8 Optimization of the extraction of formaldehyde in milk samples

The optimization of significant variables for extracting formaldehyde in milk samples was studied from a complete factorial Central Composite Rotational Design (CCRD) (16 experiments) + 6 repetitions at the central point + star points (8

experiments), totaling 30 experiments (Shenbaga et al., 2015). The variables under study were: Milk volume: 5 and 10 mL; Derivatizing solution volume: 5 and 10 mL; NaCl mass/$MgSO_4$ mass ratio: 1:2 and 1:4 and PSA mass/$MgSO_4$ mass ratio 1:1 and 1:3. Table 2 illustrates the CCRD constructed from the variables under study. The optimization was performed by fixing the amount of 0.50g of NaCl and 0.05g of PSA that would be used. The required mass of $MgSO_4$ was calculated to obtain the proportion described in table 2. In this way, weighed 0.50g of NaCl and 1.6g of $MgSO_4$ and added to the volume of 6.25 mL of milk, 6.25 mL of 10 ppm derivatized, and 200 µL of 10 ppm formaldehyde solution. The pH of this solution was controlled to 4.00 to have better derivatization. This solution was vortexed for 1 min and centrifuged for 1 min at 2400 rpm. In addition, 1.50 ml of the supernatant was taken and added to an Eppendorf. Subsequently, the amount of 0.10g $MgSO_4$ and 0.05g of PSA was weighed and added to the volume present in the Eppendorf, and the final solution was stirred for 1 min and centrifuged for 1 min at 2400 rpm. 1.00 mL of the supernatant was removed from this solution; being added to a vial, and subsequently analyzed.

2.9 Calibration curve

For construction of calibration curve in the matrix were used concentrations of 0.013, 0.053, 0.107, 0.160, 0.213, 0.267, 0.320 ppm formaldehyde. A 10ppm solution of DNPH in ACN was prepared and diluted to all concentrations of interest to perform derivatization. The optimized volume of the milk sample was measured, and this sample was fortified with formaldehyde to obtain the concentrations described above. After the fortification adding 0.50g of NaCl and 1.8g of $MgSO_4$ were. This solution was vortexed for 1 min and centrifuged for 1 min at 2400 rpm. 1.50 ml of supernatant was transferred to an Eppendorf and added 0.05g of PSA and 0.06g of $MgSO_4$, shaken for 1 min, and centrifuged at 2400 rpm. 1.00 mL of this solution was transferred to a vial and injected. Each concentration of the analytical curve was prepared and analyzed in triplicate. After performing all the injections, we obtained the calibration curve of the average areas of peaks.

2.10 Analysis of milk samples

From the CCRD used in the optimization, a better formaldehyde extraction condition was obtained to analyze milk samples (Section 2.7, Table 2). Formaldehyde extraction in milk samples occurred by adding milk volume, derivative volume, NaCl/$MgSO_4$ ratio, and PSA/$MgSO_4$ ratio according to the variables optimized by the CCRD. This procedure was repeated for all milk samples, and all were done in triplicate.

3. Results and Discussion

3.1 Physicochemical analysis

Table 1 presents the values of fat, density, freezing point (BW), protein, lactose, and solid-non-fat (SNG). When analyzing the values of fat present in the milk samples, the samples C1, F1, F2, and G2 presented the highest values. For whole milk, the minimum percentage should be 3% m/m and may vary up to 6% m/m depending on breed, diet, time of year, and lactation period (Brasil, 2018). Thus, all analyzed samples are within the required standard. In addition, the protein contents varied between 2.99% m/m and 3.31% m/m, noting that they are within the standard required by the legislation, which is at least 2.9% m/m.

Table 1 - Results of physical-chemical analysis.

LOTES	Density (g/mL)	Fat (%)	Protein (%)	PC (°C)	Lactose (%)	SNG (%)
A_1	1.0297	3.72	3.17	- 0.534	4.56	8.48
A_2	1.0299	3.87	3.23	- 0.542	4.62	8.57
B_1	1.0310	3.63	3.31	- 0.558	4.74	8.84
B_2	1.0307	3.90	3.29	- 0.557	4.73	8.79
C_1	1.0304	4.15	3.26	- 0.557	4.73	8.75
C_2	1.0297	3.99	3.18	- 0.540	4.60	8.53
D_1	1.0292	3.88	3.13	- 0.529	4.52	8.38
D_2	1.0280	3.79	3.01	- 0.506	4.35	8.06
E_1	1.0289	3.52	3.09	- 0.517	4.44	8.25
E_2	1.0291	3.69	3.12	- 0.524	4.49	8.34
F_1	1.0305	4.23	3.28	- 0.560	4.75	8.79
F_2	1.0301	4.05	3.23	- 0.549	4.67	8.65
G_1	1.0309	3.72	3.30	- 0.557	4.74	8.82
G_2	1.0291	4.42	3.13	- 0.538	4.58	8.45
H_1	1.0285	3.90	3.05	- 0.516	4.42	8.19
H_2	1.0292	3.94	3.13	- 0.530	4.53	8.39
I_1	1.0303	3.79	3.24	- 0.546	4.66	8.65
I_2	1.0304	3.82	3.26	- 0.550	4.69	8.71
J_1	1.0295	3.78	3.25	- 0.532	4.55	8.44
J_2	1.0301	3.84	3.22	- 0.544	4.63	8.60
K_1	1.0300	3.82	3.21	- 0.543	4.63	8.59
K_2	1.0302	3.94	3.23	- 0.548	4.67	8.66
L_1	1.0304	3.82	3.25	- 0.550	4.68	8.70
L_2	1.0280	3.48	2.99	- 0.499	4,30	7.99

Source: Authors.

Milk samples with protein and fat levels below or above the minimum required limit influence the quality of derivative products, resulting in prejudice for industries and rural producers, as industries pay for milk depending on the fat and protein contents (Fox and Mcsweeney, 1998). However, when analyzing the milk samples regarding the lactose content present, it was found that all were within the standard established by the legislation, which is at least 4.3% m/m.

Analysis of the samples regarding SNG values found that samples D1, D2, E1, E2, H1, H2, and L2 disagree with the current legislation that establishes a minimum content of 8.4% m/m for SNG. According with Tronco (2008), the SNG values present in milk are consistent with the milk components, minus water, and fat.

The freezing points of the milk analyzed ranged between – 0.499°C and – 0.560°C, while the current legislation establishes a margin between – 0.512°C and – 0.532°C. With these results, some brands are outside the required standard. According to Fonseca and Santos (2000), numerous factors can influence the freezing point, such as lactation stage, breed, and type of milk processing. They also add that normally values above the stipulated indicate the addition of water; however, errors in cleaning the milking and cooling equipment can also occur.

The densities ranged from 1.028 g/mL to 1.0310 g/mL. According to current legislation, the density must meet a variation between 1.028 g/mL to 1.034 g/mL. Therefore, all are within the required standards. They indicate the excellent quality of the analyzed milk. Density values below that required by legislation may indicate the addition of water or values above may indicate the addition of constituents or preservatives.

3.2 Antibiotic analysis

According to Mendes et al. (2008), antibiotic residues in milk make it unfit for consumption and a risk to public health, causing hypersensitivity reactions, induction of bacterial resistance, optic, hepatic, and renal lesions (Lozano and Arias, 2008). The results of milk samples analyzed here are within what is established (Brasil, 2018).

Qualitative analyses performed with the rapid test are essential because they could indicate which samples would disagree with the legislation. Thus, milk without antibiotic residues indicates good disease prevention, reasonable control of the disposal of contaminated milk, and respect for the grace period of each drug (Pesca et al., 2020).

3.3 Methodologic optimization for extracting formaldehyde from UHT milk

Optimization aimed to find and ensure efficiency in the derivatization of formaldehyde with DNPH and extract this analyte from milk samples. The CCRD showed that the salts' interactions are significant for the system. (Prestes, Adaime, and Zanella, 2011).

Table 2 illustrates the coded and decoded data from the CCRD used in the Optimization. In the last column are the areas of samples analyte. The design results made it possible to determine the regression coefficients for the response (Y) and build the response surfaces. The Optimization showed that the salts and milk volume ratios significantly affected the system according to the t-test (α=0.05). These results made it possible to build a significant quadratic model by multiple linear regression.

The fitted model equation determined is:

$$Y = -101677 + 22462\ x^2 - 8294\ x^2 + 164874\ x^3 + 132286\ x^4 - 1546\ x^{12} - 497\ x^{22} - 243274\ x^{32} - 31641\ x^{42} + 31641\ x^2\ x^3 + 7366\ x^2\ x^4 - 381930\ x^3\ x^4$$

Table 2 - Codified and decoded data from CCRD planning with their respective areas.

Experiment	x1	x2	x3	x4	Milk volume	Derivatizer volume	Ratio $NaCl/MgSO_4$	Ratio $PSA/MgSO_4$	Areas
1	-1	-1	-1	-1	6.25	6.25	0.3125	0.50000000	15903
2	1	-1	-1	-1	8.75	6.25	0.3125	0.50000000	9611
3	-1	1	-1	-1	6.25	8.75	0.3125	0.50000000	7929
4	1	1	-1	-1	8.75	8.75	0.3125	0.50000000	11760
5	-1	-1	1	-1	6.25	6.25	0.4375	0.50000000	10356
6	1	-1	1	-1	8.75	6.25	0.4375	0.50000000	13255
7	-1	1	1	-1	6.25	8.75	0.4375	0.50000000	21376
8	1	1	1	-1	8.75	8.75	0.4375	0,50000000	15292
9	-1	-1	-1	1	6.25	6.25	0.3125	0.833333333	20373
10	1	-1	-1	1	8.75	6.25	0.3125	0.833333333	20787
11	-1	1	-1	1	6.25	8.75	0.3125	0.833333333	25815
12	1	1	-1	1	8.75	8.75	0.3125	0.833333333	20904
13	-1	-1	1	1	6.25	6.25	0.4375	0.833333333	6536
14	1	-1	1	1	6.25	6.25	0.4375	0.833333333	-
15	-1	1	1	1	8.75	8.75	0.4375	0.833333333	15769
16	1	1	1	1	6.25	875	0.4375	0.833333333	16995
17	-2	0	0	0	8.75	7.50	0.3750	0.666666667	18128
18	2	0	0	0	5.00	7.50	0.3750	0.666666667	15016
19	0	-2	0	0	10.00	5.00	0.3750	0.666666667	25147
20	0	2	0	0	7.50	1.00	0.3750	0.666666667	21108
21	0	0	-2	0	7.50	7.50	0.2500	0.666666667	27141
22	0	0	2	0	7.50	7.50	0.5000	0.666666667	17721
23	0	0	0	-2	7.50	7.50	0.3750	0.333333333	26018
24	0	0	0	2	7.50	7.50	0.3750	1.000000000	19415
25	0	0	0	0	7.50	7.50	0.3750	0.666666667	23045
26	0	0	0	0	7.50	7.50	0.3750	0.666666667	20161
27	0	0	0	0	7.50	7.50	0.3750	0.666666667	17428
28	0	0	0	0	7.50	7.50	0.3750	0.666666667	25089
29	0	0	0	0	7.50	7.50	0.3750	0.666666667	23203
30	0	0	0	0	7.50	7.50	0.3750	0.666666667	7985

Source: Authors.

Table 3 shows the ANOVA for the model obtained. The explained variation for the model was 61.52%. Considering the adjusted model, the maximum point in CCRD to obtain maximum analyte area occurs under the following conditions: Milk volume equal to 7.3; the volume of derivative equal to 7.3; $NaCl/MgSO_4$ ratio equal to 0.28; $PSA/MgSO_4$ ratio equal to 0.88.

The estimated area at the maximum point = 29837.03, thus providing the best condition for the derivatization and extraction of milk samples.

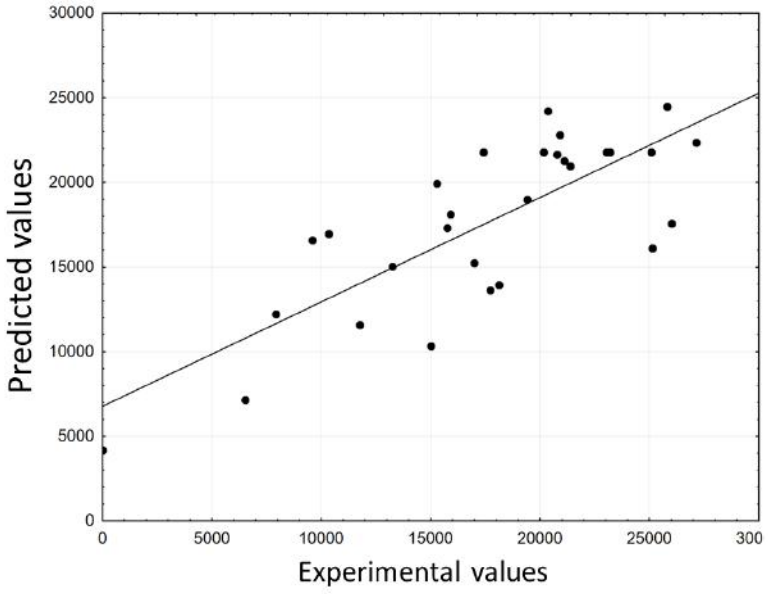
Table 3 - ANOVA table for the quadratic mathematical model obtained by the CCRD design to optimize the experimental conditions of the formaldehyde extraction system from milk samples. SV = Source of variation, QS = quadratic sum, nDG = degree number.

SV	QS	nDG	QM	*F*calc		*p*
Regression	727520404	11	66138219	2.47	SG	0.046
waste	455006469	17	26765086			
lack of fit	418870904	13	32220839	3.57		0.046
pure error	36135565	4	9033891			
Total	1182526873	28				
% explained variation				61.52%		
% maximum explainable variation				96.64 %		

Source: Authors

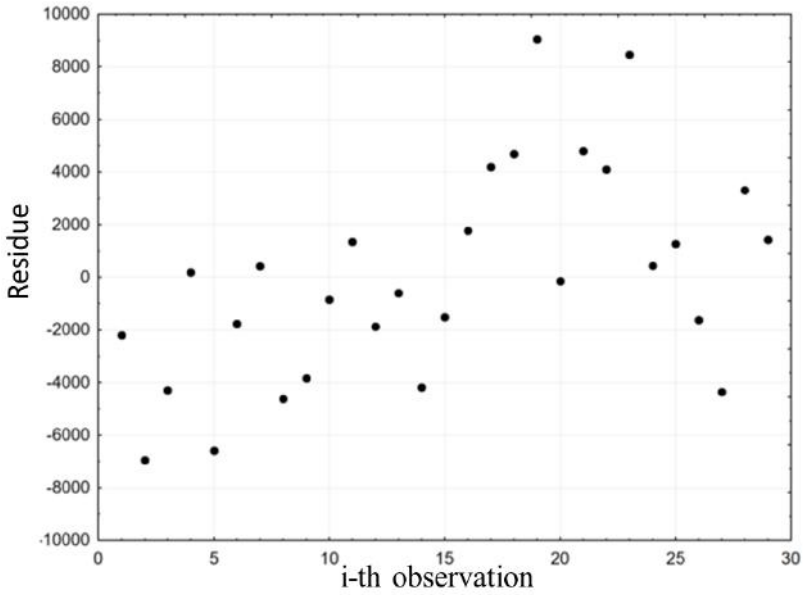
Figure 1 shows the values observed experimentally versus the values predicted by the model. There is a good agreement between them. Already, Figure 2 shows the residual graph of the model. Through the analysis of residuals, it was possible to estimate the mean squared error of the regression. Figure 2 illustrates the order of magnitude of the deviations, which was considered high compared to the order of magnitude of the response variable. Because the model explains only 61.5% of the total variation, this value corresponds to the coefficient of determination (R^2).

Figure 1 - Experimental values versus values predicted by the model for the Y response (chromatographic area).



Source: Authors.

Figure 2 - Residual plot versus i-th observation.



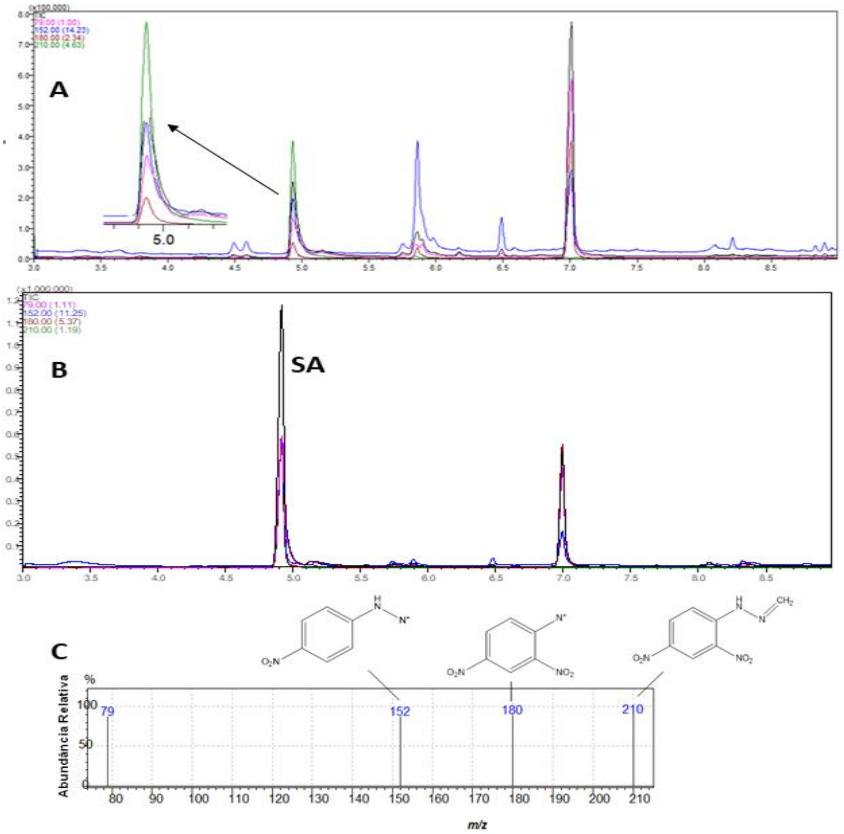
Source: Authors.

The significance level used in the tests was 5%. Even though the deviation was high, and the coefficient of determination was relatively low, the model is satisfactory for describing the response as a function of the four factors within the tested range, being significant by ANOVA of the regression.

3.4 Development of analytical methodology for detection of formaldehyde in milk

Due to milk being a complex matrix, the construction of the calibration curve occurred in the matrix, presenting the equation y=94944x+6605.7 and $R^2 = 0.99$. To monitor derivatized formaldehyde (Figure 3) in the mass spectrometer, the ion m/z 180 proved to be selective for formaldehyde after the previous testing with the analytical standard. Enabling to analyze of the milk samples using the CCRD and described chromatographic conditions. Figure 3 shows the chromatogram and mass spectrum of the milk sample A1.

Figure 3 - (A) Chromatogram of the A1 milk sample. The peak at 4.95 min is for formaldehyde-DNPH, and at 7 min is DNPH, with highlight the peak referring to the ion m/z 180. (B) Chromatogram of the standard 10 ppm formaldehyde (AS) with the retention time of 4.95 min and the DNPH with the retention time of 7 min. (C) Mass spectrum of sample A1 illustrating its ions.



Source: Authors.

According to data from the European Food Safety Authority (EFSA), to be considered endogenous levels of formaldehyde in milk samples, the content must be between 0.1 – 0.8 ppm. Values above the allowed can be considered fraudulent. The analyzed samples in this work are within the pre-established content by EFSA (Table 4).

Table 4 - Means of concentrations accompanied by the t-test result (nullity hypothesis: mean equal to zero; alternative hypothesis: mean different from zero).

	Lot 1	**Lot 2**
A	0.0391^{ns}	0.2972*
B	0.0374 ^{ns}	0.0399 ^{ns}
C	0.0371 ^{ns}	0.0292 ^{ns}
D	0.0390 ^{ns}	0.0930 ^{ns}
E	0.2898*	0.0163 ^{ns}
F	0.0461 ^{ns}	0.1223*
G	0.0416 ^{ns}	0.0441 ^{n}
H	0.0263 ^{ns}	0.0393 ^{ns}
I	0.2340*	0.0307 ^{ns}
J	0.0842 ^{ns}	0.0340 ^{ns}
K	0.0581 ^{ns}	0.0476 ^{ns}
L	0.0206 ^{ns}	0.0293 ^{ns}

Source: Authors.

Brazilian legislation does not establish minimum values for the presence of formaldehyde in foods of animal origin, and the only test they require is a qualitative one that does not allow the quantification of the formaldehyde content. Therefore, techniques such as the one developed in the present work using GC-MS are essential because they identify and quantify formaldehyde levels in milk samples. Thus, the development method provides quantitative results, unlike the method used by the Brazilian control agency.

4. Conclusion

The physicochemical and antibiotic analyses of UHT milk in this work showed results required by current legislation and proved that producers are meeting all established standards. Twenty-four milk samples were analyzed, and all presented values within limits established by the European agency since Brazil does not provide values. The results demonstrate that the optimized method for analyzing formaldehyde in milk is advantageous because it is efficient, consumes less solvent volume, is easy to reproduce, and the analysis time is short. Finally, the method developed is advantageous compared to the official method used by the Brazilian control agency. That uses a qualitative test to evaluate the presence of formaldehyde, which can be inconclusive because it is impossible to distinguish the presence of formaldehyde of endogenous origin from that of contaminant origin. In addition, this study showed a new perspective in method creation for quantifying formaldehyde in consumed milk in Brazil and allows technicians and researchers to adapt and use the method reported here.

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