Original Research Article

Optimization of Water Cooking of Potato (*Ipomea batatas*) Leaves and Characterization of Three Nutritional Interest Molecules (folic acid, iron and phytate)

ABSTRACT

Potato (*Ipomea batatas*) leaves are among the leafy vegetables most consumed by Ivorian population. In order to preserve losses of iron and folic acid, and to eliminate phytates, a study of optimization of water cooking of these leaves was conducted. Response surface methodology was employed to describe the effects of cooking time and leaf quantity on iron, folic acid and phytate contents of potato leaves using a central composite design. Response surfaces and isoresponse curves were plotted to visualize areas of interest (optimal points). Results showed that the experimental data were adequately fitted into the second-order polynomial model. Cooking time had significant effects ($P < .05$) on folic acid and phytate contents. The effect of leaf quantity was significant ($P < .01$) on the three response variables. In addition, the optimal points were located in areas of the experimental domain where iron and folic acid contents were high. Therefore, three optimal conditions for water cooking (cooking time, leaf quantity) were identified: (10 min, 400 g), (7.93 min, 300 g) and (22.07 min, 441.4 g). Under these conditions, iron and folic acid contents (mg/100g) were: (49.17, 12.58), (37.00, 16.27) and (48.77, 11.26), respectively. These results could be exploited to formulate iron and folic acid supplementation products from potato leaves.

Keywords: Potato leaves; water cooking; optimization; folic acid.

1. INTRODUCTION

The potato (*Ipomea batatas*) leaves are among the most consumed leafy vegetables in Ivory Coast [1]. They contain important sources of micronutrient including: minerals, vitamins and dietary fiber [2,3]. Among vitamins and minerals, folic acid (vitamin B9) and iron are nutritionally important. Indeed, their deficiencies would cause anemia (iron deficiency and megaloblastic anemias), as well as congenital malformations or cancers and Alzheimer's disease [4,5]. In addition, leafy vegetables help to fight against the infant-juvenile mortality, and contribute to the improvement of health state of population [6].

A preliminary investigation revealed that in Ivory Coast, potato leaves are most often water-cooked. The conditions of water cooking (water quantity, leaf quantity, cooking time, etc.) would favor micronutrient losses, in particular iron and folic acid. Indeed, Rocca-poliméni [7] has shown that cooking causes losses in certain nutrients, either by the diffusion of water-soluble constituents in the cooking water, or by the destruction of thermolabile substances.

Previous work has focused on cooking leafy vegetables. In a study conducted on three leafy vegetables consumed in Benin, Vodouhe et al. [8] found that water cooking preserved macronutrients better, while steam cooking preserved minerals better. However, this study didn't allow evaluating the effect of cooking conditions, because each cooking mode had been subjected to fixed conditions by type of leafy vegetable. Zoro et al. [3] studied the water cooking of five leafy vegetables consumed in western Ivory Coast. These authors recommended a cooking time of less than 15 min to preserve nutritional properties of the studied leafy vegetables. However, this study didn't allow determining the exact conditions for water cooking to reduce micronutrient losses. Like these two studies, most of the work isn't concerned with determining the optimum conditions for water cooking of potato leaves.

To optimize a process by locating the optimum of experimental conditions, response surface methodology was often used. For fitting quadratic polynomial, the five-level central composite design [9] is a better alternative to the full factorial three-level design because its performance is comparable at lower cost. Response surface designs are easily applied to optimize variables [10,11]. They need fewer experiments, which are more efficient and can move through the experimental domain. Multivariate designs, which allow the simultaneous study of several control variables, are faster to implement and more cost-effective than traditional univariate approaches [12,13].

Therefore, optimization by response surface methodology appears as an interesting alternative to improve the water cooking of potato leaves. In this work, central composite design was applied to investigate the effects of cooking time and leaf quantity on iron, folic acid and phytate contents of potato leaves.

2. MATERIAL AND METHODS

2.1 Biological Material

Potato (*Ipomea batatas*) leaves were collected from traders of Gouro market in Adjamé (Abidjan, Ivory Coast). This market is a wholesale market for foods of plant origin. Then rotten leaves, leaf debris and foreign bodies were removed by hand sorting. Finally, the leaves in good condition were used for experimentation.

2.2 Methods

2.2.1 Experimental design

Optimization of water cooking of potato leaves was carried out using a central composite design [9]. The factors chosen were cooking time and leaf quantity. Experimental domain was defined according to preliminary results as follows:

- cooking time: central point 15 min, step of variation 5 min;
- leaf quantity: central point 300 g, step of variation 100 g.

Table 1 presents the levels of factors in the experimental domain.

Table 1. Experimental domain

Coded values	Cooking time (min)	Leaf quantity (g)
-1.414	7.93 (7 mn 55.8 s)	158.6
-1.000	10	200
0.000	15	300

+1.000	20	400
+1.414	22.07 (22 mn 4.2 s)	441.4

The number of experiments required (N) was determined by $N = 2^k + 2k + n_0$, where k is the number of factors and n_0 is the number of experiments at the center of the domain. For two factors and eight central points, sixteen (16) experiments were necessary. Table 2 presents the experimental matrix and design of the water cooking conditions.

Table 2. Experimental matrix and design of the water cooking conditions of potato leaves

Tests	Experimental matrix		Experimental design	
	X_1	X_2	Cooking time (min)	Leaf quantity (g)
1	-1	-1	10	200
2	+1	-1	20	200
3	-1	+1	10	400
4	+1	+1	20	400
5	-1.414	0	7.93	300
6	+1.414	0	22.07	300
7	0	-1.414	15	158.6
8	0	+1.414	15	441.4
9	0	0	15	300
10	0	0	15	300
11	0	0	15	300
12	0	0	15	300
13	0	0	15	300
14	0	0	15	300
15	0	0	15	300
16	0	0	15	300

X_1 and X_2 are coded values of cooking time and leaf quantity respectively

2.2.2 Process for cooking potato leaves

The healthy leaves were weighed according to the experimental design (Table 2). They were cut, washed in drinking water and drained to remove dust and chemical residues. Then, 500 ml of water was heated in a stainless steel pan using a hot plate (200 W, SEVERIN, Illkirch Graffenstaden, France) set at 100 °C. A thermometer was introduced into the covered pan at 3/4. As soon as the water began to boil (≈ 95 °C), the thermometer was removed and the potato leaves, previously cleaned, were introduced into the pan. The cooking time was then programmed according to the experimental design (Table 2) and the pan remained covered at 3/4. Finally, the potato leaves were drained at room temperature (20 °C) and oven-dried (BOV-V125F, BIOBASE, Jinan, China) for 72 h at 45 °C. Once dry, the potato leaves were milled using a blender and stored in the freezer at -4 °C in airtight containers for subsequent analysis.

2.2.3 Determination of experimental responses

The experimental responses were iron, folic acid and phytate contents. Iron content was assayed by atomic absorption spectrophotometry (SpectrAA, VARIAN, Palo Alto, USA) according to the AOAC [14] digestion method using strong acids. Folic acid content was determined by high performance liquid chromatography (Nexera, SHIMADZU, Kyoto, Japan)

according to the method developed by El-Gizawy et al. [15]. The stationary phase was a cyclobond I column. The mobile phase was a methanol/phosphate buffer (20:80) solution at pH 7. Phytate content were quantified by UV/VIS spectrometry (Rayleigh, Beifen-Ruili, beijing, China) according to the method described by Latta and Eskin [16], based on the decoloration of the Wade reagent by phytates.

2.2.4 Statistical analyzes of the data

A second-order polynomial regression model with six coefficients (b_0, b_1, b_2, b_{11}, b_{22}, b_{12}) was used to express Y as a function of the factors as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (1)$$

where Y represents the response variables, X_1 and X_2 are the coded values of the factors.
To determine factor effects and model coefficients, multiple regression analysis and analysis of variance were performed. The statistical significance test was based on the total error criteria with a confidence level of 95.0 %. To optimize the responses, the coordinates of the stationary points of the response surfaces were calculated by differentiating the equations of the responses with respect to each variable and solving the following system of equations [17]:

$$\begin{cases} \delta Y/\delta X_1 = 0 \\ \delta Y/\delta X_1 = 0 \end{cases} \Longrightarrow S = (Xs_1; Xs_2) \quad (2)$$

where S is the stationary point and, Xs_1 and Xs_2 are its coordinates in the experimental domain.
The distance from the stationary point to the center of the experimental domain (D_S) was then determined as follows:

$$D_S = [(Xs_1)^2 + (Xs_2)^2]^{1/2} \quad (3)$$

Since the stationary points weren't the desired optimal points, response surface and isoresponse curves were generated using the second-order polynomial model. These graphs were visualized to identify areas of interest. All statistical analyzes and plots were made using Statistica 7.1 software [18].

3. RESULTS AND DISCUSSION

3.1 Analysis of Experimental Results

Experimental responses and phytate/iron ratios obtained from the experiments are shown in Table 3.

Iron content varied between 20 and 49.17 mg/100g. These contents were higher than those, ranging from 15.44 to 29.90 mg/100g, obtained by Zoro et al. [3]. These authors cooked the potato (*Ipomea batatas*) leaves for a longer time (15 to 45 min). Therefore, this difference in results could be explained by cooking time. In fact, micronutrient content decreases with cooking time of the leafy vegetables [3]. Iron is an indispensable mineral in the prevention of anemia [19]. Considering a bioavailability of 15 %, the recommended iron intake for adult woman is 19.6 mg/day [20]. In view of the contents obtained, consumption of cooked potato leaves could help cover the daily iron requirement of adult women.

Folic acid content oscillated between 3.83 to 16.27 mg/100g. Folic acid plays an important role in the formation of red blood cells, the functioning of nervous system and the immune

system [21]. It also promotes the prevention of neural tube (*Spina bifida*) closure abnormalities, and cardiovascular diseases [4]. Superior Council of Health recommends a daily intake of 0.2 mg of folic acid for adult woman [22]. The consumption of cooked potato leaves could help cover the daily folic acid requirement of adult women.

Phytate content ranged from 21.67 to 48.33 mg/100g. Phytates are antinutritional substances that chelate metal ions such as iron, preventing intestinal absorption during feeding [23]. Therefore, ratio phytate/iron is an indicator of the availability of iron for the body. This ratio, ranging from 0.95 to 1.09, was greater than the critical value of 0.4 [24,25]. The iron contained in cooked potato leaves may be less available to the body. Therefore, it would be advantageous to prepare these potato leaves accompanied by proteins of animal origin (fish, meat or egg) which are activators of iron absorption [26].

Table 3. Experimental responses and phytate/iron ratios

Tests	Experimental responses			Phytate/iron ratios
	Iron content	Folic acid content	Phytate content	
1	36.08	6.30	36.36	1.01
2	33.43	3.83	32.00	0.96
3	49.17	12.58	48.33	0.98
4	41.70	4.04	44.38	1.06
5	37.00	16.27	40.33	1.09
6	34.17	5.78	34.67	1.01
7	20.00	5.03	21.67	1.08
8	48.77	11.26	46.50	0.95
9	36.22	6.31	37.00	1.02
10	36.29	6.51	37.52	1.03
11	36.27	6.81	37.91	1.05
12	36.30	6.60	38.00	1.05
13	36.27	6.58	37.67	1.04
14	36.23	6.60	37.85	1.04
15	36.23	6.57	37.33	1.03
16	36.20	6.90	37.00	1.02

3.2 Analysis of the Model

Variance analysis of the factors studied for the response surface model is presented in Table 4. Statistical analysis showed that the regression models for the response variables were highly significant ($P < .001$). Cooking time had significant effects ($P < .05$) on folic acid and phytate contents. The effect of leaf quantity was significant ($P < .01$) on the three response variables.

Table 5 summarizes the multiple regression coefficients obtained by a least squares technique to predict the second-order polynomial model of each response variable. For iron content, examination of these coefficients, using the student's *t*-test, indicated that the linear term of leaf quantity was the only significant term ($P < .001$). For folic acid content, the linear terms of cooking time and leaf quantity, and the quadratic term of cooking time were significant ($P < .05$). With regard to phytate content, the linear terms of cooking time and leaf quantity were significant ($P < .05$). Moreover, for the three response variables, interaction wasn't significant ($P > .05$) within the experimental domain. Overall, these results suggest that the linear term of leaf quantity was the main factors affecting the three response variables.

For each response variable, the equation of second-order polynomial (1) can be written with six coefficients as follows:

$$I = 36.25 - 1.77 \times T + 7.75 \times Q - 1.20 \times T \times Q + 0.94 \times T^2 + 0.34 \times Q^2 \quad (4)$$

$$FA = 6.61 - 3.23 \times T + 1.91 \times Q - 1.52 \times T \times Q + 1.48 \times T^2 + 0.04 \times Q^2 \quad (5)$$

$$FA = 6.61 - 3.23 \times T + 1.91 \times Q - 1.52 \times T \times Q + 1.48 \times T^2 + 0.04 \times Q^2 \quad (6)$$

where T and Q represent the coded values of cooking time and leaf quantity, respectively; I, FA and P are respectively iron, folic acid and phytate contents (mg/100g).

The coefficients of determination (R^2) were 83 %, 88 % and 90 % for iron, folic acid and phytate contents, respectively. This means that the regression models for the response variables were satisfactory. Indeed, according to Guan and Yao [27], fit of a model is good when coefficient of determination is greater than 80 %.

3.3 Determination of the Stationary Points Coordinates

The stationary points coordinates and their corresponding experimental values are presented in Table 6.

For iron and phytate contents, the distances (Ds) from stationary points to the center of the experimental domain were greater than 1.414; which meant that their stationary points were outside the experimental domain. Therefore, they couldn't be used to determine optimal parameters. As for folic acid content, Ds was less than 1.414; its stationary point was inside the experimental domain. In addition, the coded coordinates of the stationary point were (1.27, 0.36) for folic acid content. Converted to non-coded values, they gave (21.38 min, 335.88 g). At this stationary point, the predicted value of folic acid content was 4.89 mg/100g. This value was too low and not appropriate because one of the objectives was to maximize folic acid content. Therefore, this stationary point wasn't the optimal point desired.

3.4 Exploitation of Response Surfaces and Isoresponse Curves

The visualization of the response surfaces and isoresponses curves allowed to follow the evolution of the factors and their influence on the response variables, as well as to locate the areas of interest.

Table 4. Analysis of variance for response surface models

Variables	df	Iron content				Folic acid content				Phytate content			
		SS	SA	*F*	*P*	SS	SA	*F*	*P*	SS	SA	*F*	*P*
Model	5	519.80	103.96	10.03	.001	139.65	27.93	14.33	< .001	488.15	97.63	17.54	< .001
T	1	24.94	24.94	2.41	.15	83.54	83.54	42.87	< .001	33.28	33.28	5.98	.03
Q	1	480.98	480.98	46.39	< .001	29.27	29.27	15.02	.003	442.17	442.17	79.45	< .001
T*Q	1	5.80	5.80	0.56	.47	9.20	9.20	4.72	.055	0.04	0.04	0.01	.93
T^2	1	7.14	7.14	0.69	.43	17.63	17.63	9.05	.013	9.72	9.72	1.75	.22
Q^2	1	0.95	0.95	0.09	.77	0.01	0.01	0.01	.93	2.94	2.94	0.53	.48
Residues	10	103.68	10.37	-	-	19.49	1.95	-	-	55.65	5.57	-	-

Note. T: cooking time (min); Q: leaf quantity (g); df: degree of freedom; SS: sum of squares; SA: square averages; Fisher's F test set at P ≤ .05.

Table 5. Effects of the factors on the dependent variables and coefficients of the 2nd degree model

Terms	Iron content				Folic acid content				Phytate content			
	Coefficient	Standard deviation	*t*	*P*	Coefficient	Standard deviation	*t*	*P*	Coefficient	Standard deviation	*t*	*P*
β_0	36.25	1.14	31.84	< .001	6.61	0.49	13.39	< .001	37.53	0.83	45.00	< .001
T (β_1)	-1.77	1.14	-1.55	.15	-3.23	0.49	-6.55	< .001	-2.04	0.83	-2.45	.034
Q (β_2)	7.75	1.14	6.81	< .001	1.91	0.49	3.88	.003	7.43	0.83	8.91	< .001
T*Q (β_{12})	-1.20	1.61	-0.75	.47	-1.52	0.70	-2.17	.055	0.10	1.18	0.09	.93
T^2 (β_{11})	0.94	1.14	0.83	.43	1.48	0.49	3.01	.013	1.10	0.83	1.32	.22
Q^2 (β_{22})	0.34	1.14	0.30	.77	0.04	0.49	0.09	.93	-0.61	0.83	-0.73	.48
R^2	83 %				88 %				90 %			
R^2 adjusted	75 %				82 %				85 %			

T: cooking time (min); Q: leaf quantity (g); R^2: coefficient of determination; student's t-test set at P ≤ .05.

Table 6. Stationary points

Stationary points				Corresponding experimental values			
Coord.	**Iron**	**Folic acid**	**Phytate**	**Factors**	**Iron**	**Folic acid**	**Phytate**
Xs_1	50.10	1.27	0.65	T (min)	265.51	21.38	18.24
Xs_2	77.02	0.36	6.14	Q (g)	8001.73	335.88	914.33
Ds	91.88	1.32	6.18	Response (mg/100g)	290.35	4.89	59.69

Ds: distance from stationary point to the center of the experimental domain; Xs_1 and Xs_2: coordinates of the stationary point; T: cooking time; Q: leaf quantity.

Fig. 1 shows the effect of cooking time and leaf quantity on the iron content of potato leaves. The iron content increased with the increment of leaf quantity; while cooking time didn't have a significant effect. It seems that water cooking would have preserved the iron content of potato leaves. This could be explained by the fact that, during cooking, a small quantity of water (500 ml) was used. Also, iron migration in cooking water has been limited [28]. Indeed, Nafir et al. [29] claimed that since minerals are water soluble, spinach leaves should be cooked in a very small quantity of water to minimize losses.

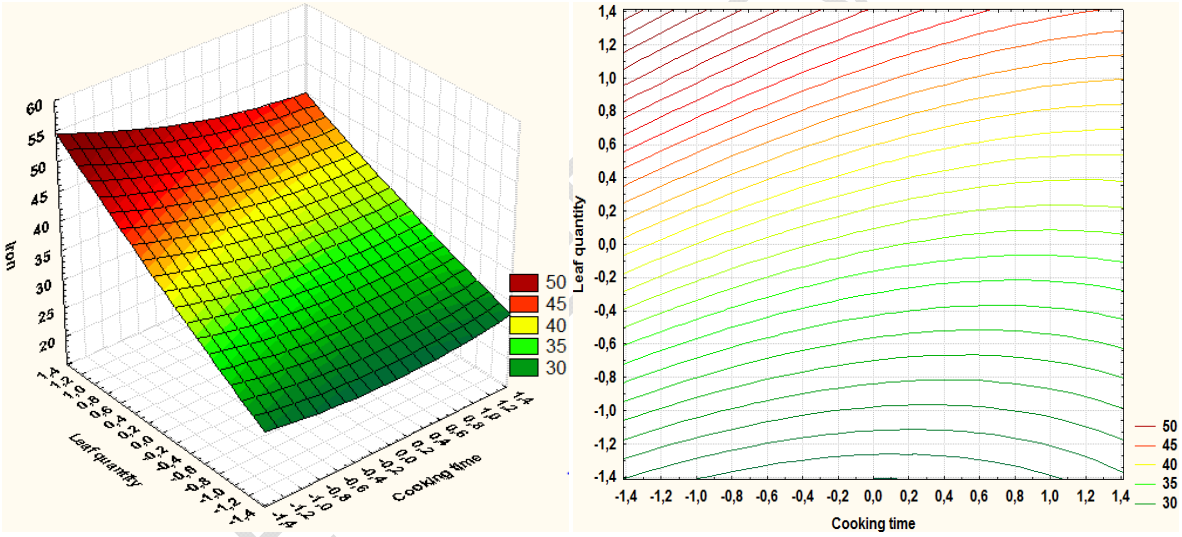

Fig. 1. Response surface and isoresponse curves: effect of cooking time and leaf quantity (in coded value) on iron content (in mg/100g) of potato leaves

The effect of cooking time and leaf quantity on the folic acid content of potato leaves can be seen in Fig. 2. The folic acid content decreased with the increasing cooking time and the reducing leaf quantity. In other words, cooking time and leaf quantity had an influence on the folic acid content of potato leaves. However, the effect of cooking time was the most significant. Water cooking resulted in losses of folic acid. Indeed, during the cooking of potato leaves, part of folic acid was destroyed by the heat emerging from the cooking water and other part diffused in the cooking water. Thus, the loss of folate would have been the result of the combination of two mechanisms that are thermal degradation and leaching of folates in the cooking or bleaching liquids [30]. In addition, the loss of folate by the diffusion phenomenon would be more marked, when the volume/surface ratio is high [31].

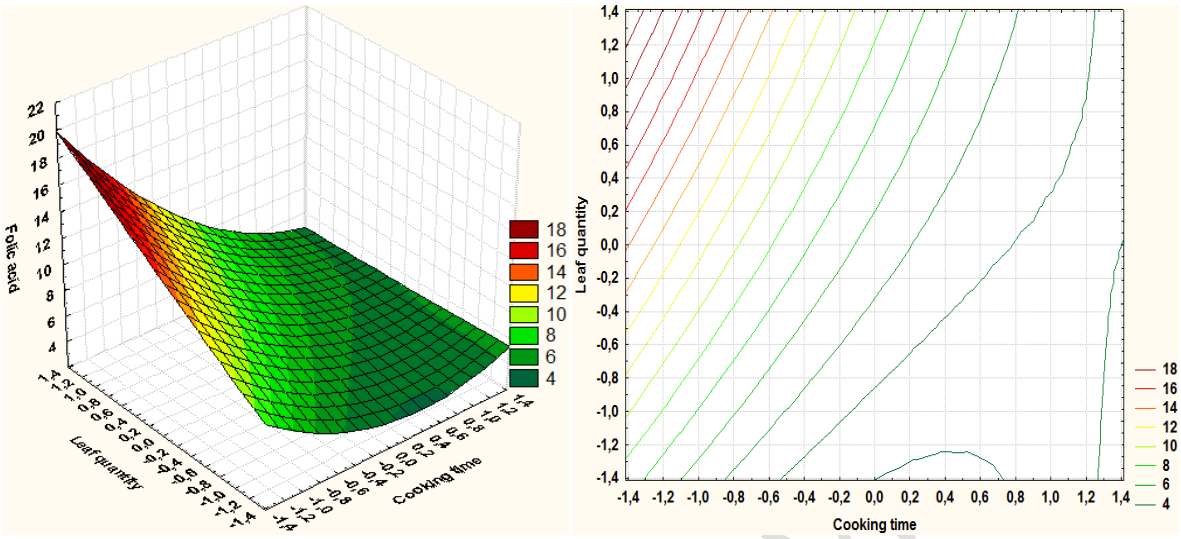

Fig. 2. Response surface and isoresponse curves: effect of cooking time and leaf quantity (in coded value) on folic acid content (in mg/100g) of potato leaves

Fig. 3 illustrates the effect of cooking time and leaf quantity on the phytate content of potato leaves. The phytate content decreased with the increasing cooking time and the reducing leaf quantity. However, leaf quantity had the most significant effect. The water cooking of potato leaves resulted in phytate losses. The losses observed could be explained by the diffusion of phytates into the cooking water [32]. Thus, cooking of potato leaves makes it possible to reduce these substances. As a result, it appears as a detoxification process [3]. Nevertheless, the results showed that the phytate/iron ratio, ranging from 0.95 to 1.09, was above the critical value of 0.4 [24,25]. In addition, the effect of cooking time was significant on the phytate content, while it wasn't on the iron content. Therefore, phytate/iron ratio could be further reduced by increasing cooking time while taking into account the sensitivity of folic acid.

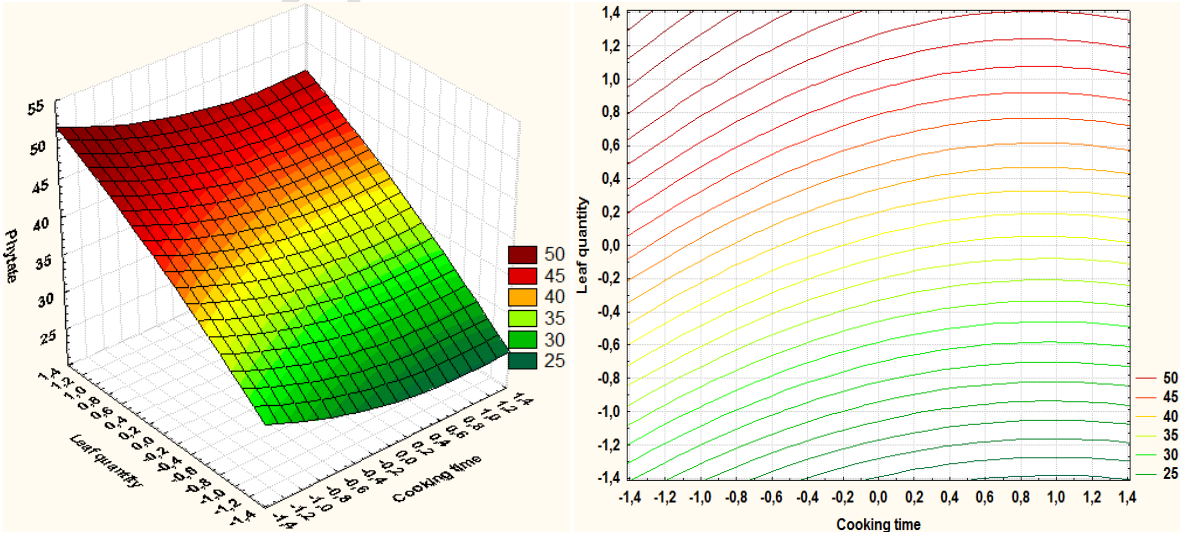

Fig. 3. Response surface and isoresponse curves: effect of cooking time and leaf quantity (in coded value) on phytate content (in mg/100g) of potato leaves

The optimization was aimed at maximize iron and folic acid contents. According to the experiment (Table 3), the experimental conditions maximizing these contents were (10 min, 400 g), (7.93 min, 300 g) and (15 min, 441.4 g). The experimental responses, in mg/100g, were respectively (iron 49.17, folic acid 12.58), (iron 37, folic acid 16.27) and (iron 48.77, folic acid 11.26).

4. CONCLUSION

The second order polynomial model is sufficient to describe and predict response variables - iron, folic acid and phytate contents of potato leaves- by considering cooking time and leaf quantity as factors. In the experimental domain, cooking time significantly influenced the folic acid and phytate contents; while leaf quantity significantly affected the three response variables. Overall, the results suggest that leaf quantity is the main determining factor affecting the three response variables. In addition, the optimal points were located in areas of the experimental domain where iron and folic acid contents were high. Therefore, three optimal conditions of water cooking (cooking time, leaf quantity) could be exploited to formulate iron and folic acid supplementation products from potato leaves.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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