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Modeling of nitrate leaching kinetics during Spinach Leaf Midribs blanching

Omar AOUN [1,2] *, Salem BENAMARA [3], Farid DAHMOUNE [1,4], Hocine REMINI [1,4]
Sofiane DAIRI [1,5], Amine BELBAHI [1,6], Brahim BOUSALHIH [2], and Khodir MADANI [1]

[1] Laboratoire Biomathématiques, Biophysique, Biochimie et de Scientométrie (L3BS). Faculté des Sciences de la Nature et de la Vie, Université de Bejaia. 06000 Bejaia, Algérie. [2] Laboratoire de recherche (E.R.P), Faculté des Sciences de la Nature et de la Vie et des Sciences de la Terre, Université de Khemis Miliana, 44225 Khemis Miliana, Algérie. [3] Département de Technologie Alimentaire, FSI, Université de Boumerdès, 35000, Algérie. [4] Département de Biologie, Faculté des Sciences de la Nature et de la Vie et des Sciences de la Terre, Université de Bouira, 10000 Bouira, Algérie. [5] Département de Microbiologie Appliquée et Sciences Alimentaires, Faculté des Sciences de la Nature et de la Vie, Université de Jijel, 18000 Jijel, Algérie. [6] Faculté des Sciences, Université de M'sila, 28000 M'sila, Algérie.

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ABSTRACT

Background: Although nitrates, are sometimes favorable to health, they can however convert to nitrosamines inside the body thanks to the acidic medium of gastrointestinal tract. So, the investigation of the nitrate content in food products becomes an imperative since it allows consumers to choose their food deliberately. **Aims:** The leaching kinetics of nitrates during water blanching of spinach leaf midribs (SLM) was investigated at different conditions of time and temperature. **Methods and Material:** The nitrate leaching kinetics, during the water blanching of SLM samples, was studied at 60, 70 and 80 ° C; for 3 and 15 minutes. Presently, six models, namely Henderson and Pabis, logarithmic, zero order, Lewis, Page, Wang, and Singh were tested to analyze experimental data. Moreover, to elucidate the effect of the temperature on the nitrate diffusion rate, the equation of Arrhenius was applied. **Results:** Results showed that after 15 min of blanching, the removal rate (RR) of nitrates was of: 23.851±3.477c, 64.809±0.474b and 75.949±5.366a % at 60, 70 and 80 °C, respectively; with a significant difference between values at (p≤0.05). Furthermore, among the six tested models, the logarithmic model seemed to be the most appropriate ($R^2 > 0.993$) to describe the diffusion kinetics of nitrates from food matrix into the blanching water, whatever the processing temperature. Finally, the activation energy (35.76 kJ. Mol^{-1}), characterizing the nitrate leaching, was assessed based on the rate constant appearing in the most appropriate model. **Conclusions:** Blanching in water constitutes an effective tool for controlling the nitrate content in vegetables, by varying the time and temperature of treatment.
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* Corresponding author   Tel: +213 775882280   omar.aoun@univ-bejaia.dz / aoun.omar@yahoo.fr

1   INTRODUCTION

The European Food Safety Authority (EFSA, 2010) has alerted about the possible existence of methemoglobinemia risk in children, due to overconsumption (>1 portion a day) of spinach [1]. Although, in recent years, a new opinion on a possible protective effect of nitrates against cardiovascular disease has been reported in the literature [2, 3], other authors continue to warn about the potential risk linked with excessive ingestion of nitrates [4, 5] and against the revision of standards of nitrate content in foods [6]. There is a lack of consensus on health risks and benefits of dietary nitrate and nitrite [7]. Actually, the metabolic effects of nitrates and other chemicals, on the body, depend, not solely on the consumed dose, but on

individuals' physiology too. Therefore, Takaki [8] showed that nitric oxide (NO) seemed to be inversely associated with fatigue among women. Furthermore, it reduces the association between job strain and fatigue. Aiming to investigate health risk assessment of nitrate contamination in groundwater in Northeast China, Su *et al.* [9] demonstrated that children's health risks are greater than those of adults. On the other hand, considering that the nitrate poisoning from home-prepared vegetable foods for infants continues to arise [10]. Hence, it is essential to investigate the way to reduce the level of nitrates in food product downstream of the harvest period and marketing. Blanching process, as a unit operation is widely used in food industry and culinary arts. Acting on the improvement of cold storage of food [11], it implies moreover the elimination of undesirable substances such as pesticide residues [11, 12].

In the current study, the leaching kinetics of nitrates, during water blanching of spinach leaf midribs (SLM), was investigated at different time and temperature conditions. To the best of our knowledge, the effect of blanching, on nitrate loss, is studied only in the case of whole spinach [13]. It should be noticed too that midribs are known to accumulate more nitrates than leaf lamina, because of their low content in nitrate reductase and the presence of a specific nitrate transporter gene [14].

2   MATERIAL AND METHODS

2.1 Plant sample

The spinach leaf midribs (SLM), including petioles, represent the plant material used to investigate the leaching kinetics of nitrates during blanching in water. Those organs are known to accumulate about 40% of all nitrates assimilated by the whole plant [15]. It has been reported that the petioles removal is known to be a way through which nitrates could be reduced in spinach [16].

Spinach were purchased in May at Boumerdès city (50 km east of Algiers). After washing, midribs and petioles were carefully separated from leaves using a hill stainless steel. The midribs were cut into pieces of 1 cm length.

2.2 Spinach midribs blanching

As cited above, blanching, beside washing and cooking processes constitute a mean to reduce nitrates in foods [17].

The sample handling applied to study the leaching of nitrates during water blanching of SLM at 60, 70, and 80°C is shown below. It was established that the blanching, as a short heat treatment, is generally carried out at temperatures ranging from 70 to 100 °C [18].

10 g of each representative sample (SLM) were put in glass containers, and 100 ml of distilled water were added. For each treatment temperature (60, 70, and 80 ° C), five samples were placed in the same temperature for 15 min.

For the kinetic analysis of nitrate leaching, a sample collection was performed during water blanching at regular intervals of 3 min, for 15 min. For comparison purpose, nitrates were quantified simultaneously in the SLM and blanching water.

2.3 Modeling of nitrates leaching kinetic during water blanching

Several works are devoted to the investigation of various physic-chemical transformations taking place during blanching of various types of foods: French fried potatoes [19], pumpkin (*Cucurbita maxima L.*) [20], non-conventional leafy vegetables [21], and Irish York cabbage [22]. However, other previous numerous articles, about the nitrate loss during blanching, are reported in the literature and concern: Carrots [23], Spinach's [13], and Cruciferous vegetables [24]. Ekart *et al.,* [25] had communicated on the influence of food processing on nitrate levels in vegetables. As regards spinach blanching, in particular, the question is sufficiently discussed but solely from the point of view of the loss of bioactive substances. Sheetal *et al.* [26] for example investigated the stability of vitamin C and peroxidase during blanching at different conditions, including microwaves.

One way to optimize the blanching management is to conduct kinetic studies and model the temperature and time effects on the level of property changes [27]. Presently, six models, namely: Henderson and Pabis, logarithmic, zero order, Lewis, Page, and Wang and Singh (Table 1) were tested (using the Minitab software 15) to analyze experimental data related to the kinetic of nitrate leaching during water blanching of SLM. These models are those applied by Doymaz and Ismail [28] to investigate water removal during drying of sweet cherry and they are normally resulting by simplifying the general series solution of Fick's second law. Lewis, Henderson, and Pabis, logarithmic and Page models are widely used to describe water diffusion in most biological materials [29].

Considering that the investigation of nitrate leaching can be considered as an extraction procedure [13] and that the extraction curves show a similar shape to that of the sorption curves [30], it becomes possible to use the same mathematical models for describing the leaching kinetics of nitrates. The latter authors have, moreover, employed Page and logarithmic models, to analyze the process of solid-liquid extraction of polyphenols from soybeans. Studying the evolution of antiradical capacity with treat-

Table 1: Results related to the modeling of the diffusion kinetic of nitrates during water blanching of spinach leaf midribs

Model and equation	Temperature (C°)	k (min-1)	a	b	c	n	R^2
Henderson and Pabis $C/C_0 = a\exp(-kt)$	60	0.016	0.971	-	-	-	0.922
	70	0.074	0.959	-	-	-	0.980
	80	0.095	0.948	-	-	-	0.989
Logarithmic $C/C_0 = a\exp(-kt)+c$	60	0.066	0.343	-	0.638	-	0.930
	70	0.138	0.722	-	0.267	-	0.996
	80	0.137	0.813	-	0.154	-	0.996
Zero order $C/C_0 = 1(-kt)$	60	0.017	-	-	-	-	0.858
	70	0.050	-	-	-	-	0.851
	80	0.058	-	-	-	-	0.830
Lewis $C/C_0 = \exp(-kt)$	60	0.019	-	-	-	-	0.889
	70	0.075	-	-	-	-	0.965
	80	0.097	-	-	-	-	0.981
Page $C/C_0 = \exp(-kt^n)$	60	0.007	-	-	-	1.437	0.839
	70	0.009	-	-	-	1.948	0.850
	80	0.040	-	-	-	1.420	0.915
Wang and Singh $C/C0 = 1+at+bt^2$	60	-	0.030	0.001	-	-	0.665
	70	-	0.046	0.007	-	-	0.619
	80	-	0.100	0.004	-	-	0.920

ment time, Suh *et al.* [31] found that the zero-order kinetic model describes more adequacy experimental data. Quispe-Fuentes *et al.* [32] communicated that among several applied models, the modified Henderson and Pabis model presents the best fit quality regarding the leaching process of saponins from quinoa (Chenopodium quinoa Willd.) seeds during washing with water. Finally, experimental data, related to the kinetics and temperature dependent moisture diffusivities of Pumpkin seeds during drying, were fitted into four thin-layer drying models including Lewis as well as Wang and Singh models [33].

Furthermore, to elucidate the effect of the temperature on the diffusion rate, the Arrhenius equation was applied (01):

$$k = k_0 \exp(-E/(RT)) \dots\dots\dots\dots (01)$$

Where, k = diffusion rate, min^{-1}, k_0 = pre-exponential constant, min^{-1}, E = activation energy, $kJ.mol^{-1}$, and R = 8.314×10^{-3} $kJ.mol^{-1}K^{-1}$ and T= temperature.

2.4 Nitrate determination procedure

Determination of nitrate was performed using the spectrophotometric procedure described by Golubev [34] and improved by Aoun *et al.* [35].

Fifty (50) g of representative sample was homogenized with distilled water for 30 min in volumetric flask (500ml). After homogenization, the suspension was allowed to stand for 1 hour with periodic mixing. The volume was then brought to the mark (at 500 ml) with the distilled water before being mixed and filtered. Then, 50 ml of the filtrate was acidified with 4 ml of acetic acid (2%) until pH 4. The solution was then evaporated until dry using Rotavapor (BUCHI Switzerland). The obtained residue was solubilized with a small quantity of distilled water, and the new solution obtained was filtered in a volumetric flask at 100 ml. The filtrate was supplemented with 5 ml of saturated NaCl solution and the volume was finally brought to the mark with distilled water. Four (4) ml of Diphenylamine reagent was added to 1 ml of final extract (using glass cells with optical path lengths of 1 cm).

The absorbance was measured with spectrophotometer (spectrophotometer UV-VIS/ JASCO V-530) at 590 nm. The nitrate content is deduced from a calibration curve which was traced using standard solutions (0.015, 0.030, 0.045, 0.075, and 0.150 mg KNO_3/ ml) according to the procedure reported by Aoun *et al.*, [34]

The Diphenylamine reagent was prepared by adding slowly 90 ml of concentrated sulfuric acid (98%) to 10 ml of distilled water, avoiding stirring. After that, the solution was added progressively and in small amounts to 0.5 g of Diphenylamine, according to the method described by: Bartzatt and Donigan [36], and Aoun *et al.* [35].

2.5 Statistical analysis

All statistical analyses were performed using STATISTICA program (Version 5.5). The results were expressed as mean ± SD, and a one-way analysis of variance (ANOVA) was performed. In all data analysis, a value of $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ were the signification levels tested. All tests were performed in triplicate (n=3).

The Minitab software (Version 15) was used to elucidate the modeling of the kinetics of nitrates leaching during blanching at different temperatures.

3   RESULTS

The calibration plot of nitrate concentration, as a function of absorbance (not presented here), was found to be linear in the concentration range studied (up to 0.150 mg KNO_3/ ml, with R^2 = 0.991), whereas the initial nitrate content of SLM is of (3860 ± 1.789) mg / kg.

Figure 1 shows the concentration variation of nitrates SLM during their blanching in water at different temperatures. As it can be observed, the nitrate content decreases over the blanching process whatever the temperature. However, the decrease rate dramatically increases when the temperature varies from 60 to 70°C, after which the nitrate rate diffusion takes place slowly.
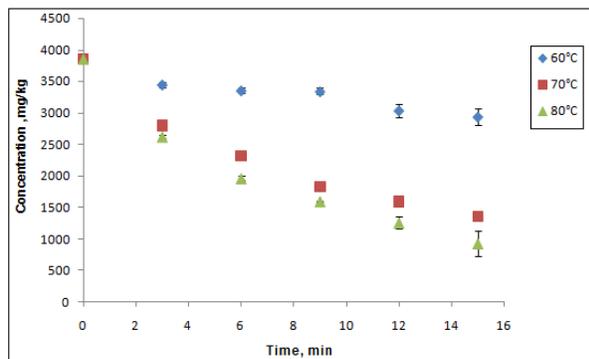

Figure 1: Variation of nitrate concentration in Spinach leaf midribs during blanching at different temperatures

From these results, are deduced removal rates (RR) of nitrate [RR = 1- (C15/C0)]; where C_0 and C_{15}: concentration of nitrates in spinach stalks at t = 0 and after 15 min of blanching, respectively.

Rates obtained at three different temperatures are displayed by the histogram on figure 2. It can be observed that the RR value at 80°C (75.949±5.366a) %, is the highest, followed by that obtained at 70°C (64.809±0.474b) %, with significant differences ($p \leq 0.05$) observed. In contrast, the RR at 60 °C was four times lower than RR 80°C (p<0.05).
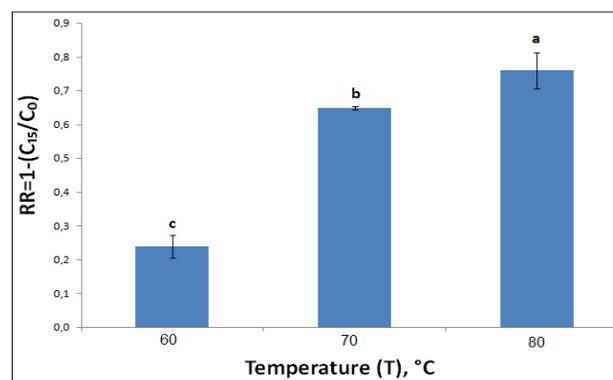

Figure 2: Removal rate (RR) of nitrates versus blanching temperature of spinach leaf midribs after 15 minutes. The Same letters indicate the absence of significant differences ($p \leq 0.05$) between values

Generally, the curve of figure 3 shows an inverse shape compared to that of figure 1 that shows that the nitrate concentration, in blanching water, varied with time and temperature of heat treatment. It is comprised between (213.167 ± 21.956) mg/l at 60 °C, after 3 minutes of treatment, and (1473.333 ± 103.545) mg/l after 15 min at 80 °C. The nitrates content, in the bleaching water, deduced after 15 minutes of bleaching at 60 °C was three times lower than that obtained at the highest values of time and temperature.
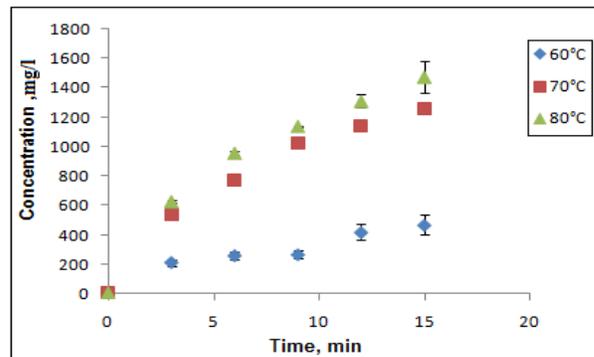

Figure 3: Variation of the nitrate content in the blanching water as a function of time and temperature of treatment

Similarly, the results on Figure 4, show that the recovery rate (RRt = C_{15}/C_0) of nitrates in the bleaching water, after 15 minutes of treatment, was considerably influenced by the treatment temperature: 12.120±1.734c, 32.599±0.234b, and 38.169±2.682a % at 60, 70, and 80 °C, respectively. A significant difference was observed (p<0.05).
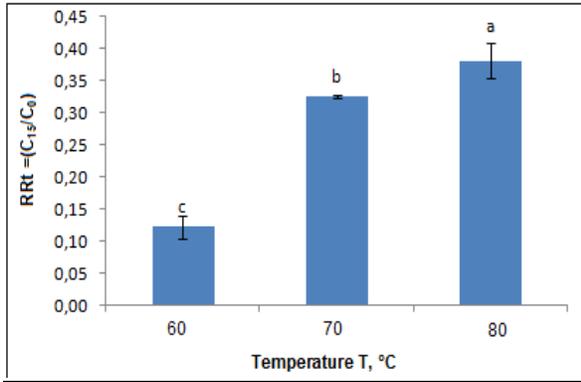

**Figure 4:** Recovery rate (RRt) of nitrates in the blanching water, after 15 min of treatment at different temperatures. The Same letters indicate the absence of significant differences (p≤0.05) between values

Table 1 summarizes the results related to the modeling of the leaching of nitrates during water blanching of SLM. The most important information contained in these data, concerns the appropriateness of the logarithmic model, provided the value of the coefficient of determination (R^2> 0.993) at 70 and 80 °C, as well as the good correlation between the values predicted by the model and those found experimentally (Figure 5).
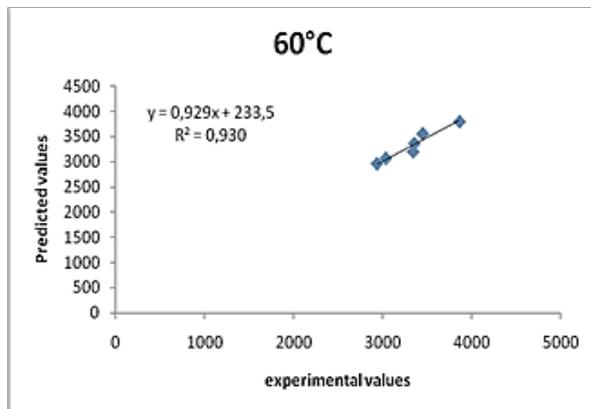
(a)
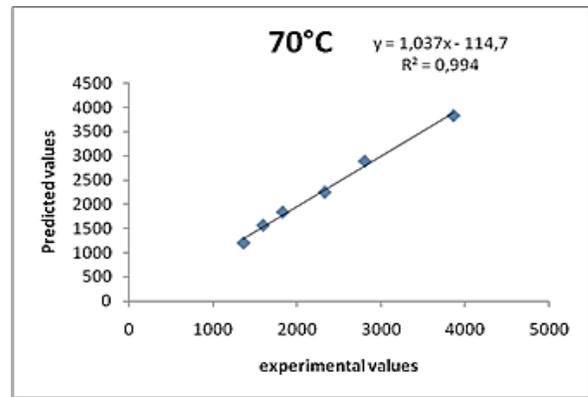
(b)
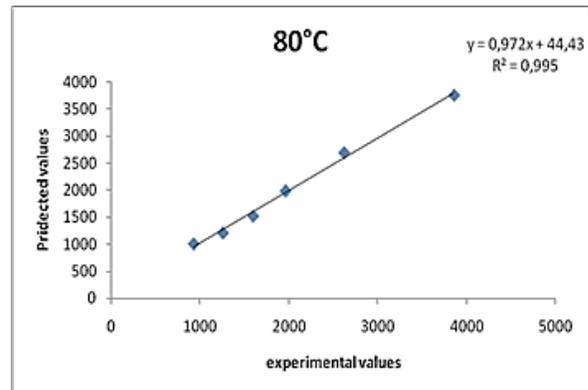
(c)

Figure 5: Correlation curves between the experimental values and the values predicted by the logarithmic model at different temperatures; a: 60, b: 70, and c: 80 °C

Figure 6 displays the effect of temperature on rate constant corresponding to the selected model. The straight slope allows to deduce the activation energy (35.76 kJ mol-1) associated with the transfer of nitrates (from the food matrix to the blanching water).
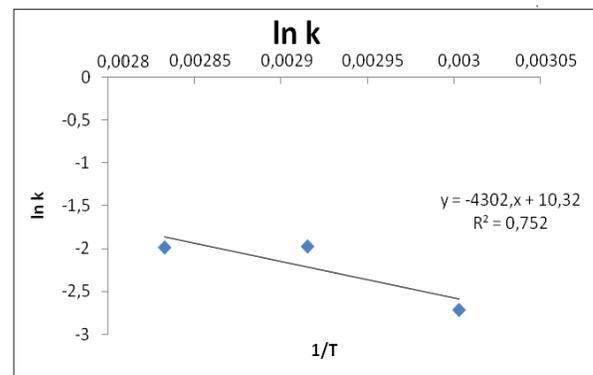

Figure 6: Correlation curve between ln(k) versus 1/T

4   DISCUSSION

The maximum level nitrates concentrations, limited by the European Union (EU Commission Regulation N° 1258/2011 2 December 2011) is of (3500 mg/Kg). Spinach, this popular leaf vegetable, known to be rich in various essential nutrients, is however susceptible to contain, at the same time, considerable amounts of nitrates [37-40]. Furthermore, it is known that the nitrate content is heterogeneously distributed in vegetable organs according the following descending order: petiole > leaf > stem > root [15]. At the same time, the petiole and midrib of wild plants present generally the similar nitrate content which is on the other hand higher than that of lamina [14], which are consistent with the value of the initial nitrate content in SLM deduced before bleaching.

We suppose that the more or less superposition of the curves at 70 and 80 °C, for both cases of SLM (Figure 1) and blanching water (Figure 3) indicates that the temperature of 70°C could be considered as critical in terms of destroying action on the tissue structure of the plant. Involving de facto subsequently an easy release of nitrate molecules from the food matrix. In fact, studying the nitrate extraction of nitrates, during blanching of spinach, Graiser *et al.* [13] reported that at temperatures lower than 57 °C, the cell membranes are impermeable to ionic substances such as nitrates and there was a unique slight difference in leaching rates between 80 and 90 °C.

The RR results (Figure 2) are consistent with those found by other authors concerning the blanching of various products: potatoes peeled, cut into halves and blanched in water at 75 °C for 20 min: 21% [41]; leaves of Hibiscus sabdariffa boiled for 10 min: 73% [42]; amaranthus (*Amaranthus cruentus*) leaves boiled for 10 min: 64% [43]; leaves of *Corchorus olitorius* cooked for 10 min: 88%. Enemo *et al.* [44] revealed that, after 15 min of blanching (at 100 ° C) of some edible plants of Niger, the rate of loss of ascorbic acid was over 90%. On the other hand, Chavarri *et al.* [45] noticed that the washing and blanching of five fruits and green vegetables, including spinach, leads to the elimination of more than 50% of pesticides.

As illustrated on Figure 4, the recovery rate (RRt) of nitrates, in the bleaching water after 15 minutes, was considerably increased exceeding 60 at 80 °C; those that justified the nitrate concentrations that lose SLM are recovered by the bleaching water Those results are in agreement with those obtained by Graiser *et al.* [13].

In addition, from the results illustrated on Figure 2 and Figure 4, we observe that, whatever the blanching temperature, the recovery rate of nitrates, in the bleaching water, was equal to almost half of the removal rate (RRt ≈ 0.5 × RR); which explains the instability of nitrates during water bleaching, and; that a significant part of the nitrates disappeared. This interesting element is to consider.

At first glance, two principal causes may explain the instability of nitrates: a) enzyme activity which has not been inhibited during heating treatment, knowing that nitrates are susceptible to the Nitrate Reductase activity [46]; and b) their interactions with other components of reaction medium (ex. carbohydrates, vitamins, amino acids, etc.), knowing that, glucose and ascorbic acid being considered as the most predisposed molecules to react with nitrate in certain conditions, including high temperatures [47,48]. This nitrates instability is supported by Ismail *et al.,* [49] who found that time and temperature stimulate considerably the oxidoreduction reactions of nitrates in blanched Amaranthus (leafy vegetable).

Results from Table 1 and Figure 5 are in concordance with those described in the literature. Thus, among the six models used for modeling the drying kinetics of apple slices, Rayaguru and Routray [50] showed that the logarithmic model is more appropriate. On the other hand, as has already been pointed out, only two studies have examined the effect of blanching on the nitrate content. Unfortunately, the kinetic modeling was not taken into consideration. It must be noticed that food blanching can, at the same time, induce other undesirable changes such as loss of water-soluble vitamins that needs to be considered for a better optimizing of the blanching process.

The value of the activation energy (35.76 kJ mol-1) associated to the transfer of nitrates from the food matrix to the blanching water was two times lower than that found (74 kJ mol-1) by Varoquaux *et al.* [23] concerning the loss of nitrate during carrots blanching. This difference could be explained by the structural nature of the two types of tissues and the difference in physicochemical composition of the two plant matrices, being that some chemical substances can influence the result of nitrate diffusion kinetics during the bleaching process [47, 48].

5   CONCLUSIONS

Our results showed that water bleaching is an effective technological operation to regulate nitrate levels in vegetables before consumption. It is worth emphasizing that nitrates are the source of nitrites as a result of their reduction in the body [51]. Therefore, we consider interesting in terms of prevention to quantify nitrates in foods before consumption in order to act on them by various treatments. Indeed, it has already been reported that the nitrate content in spinach is often 250 times higher than that of nitrites [52].

Furthermore, the removal rate of nitrates depends on the temperature of the heat treatment that it is three times higher at temperature 80 °C compared with the result obtained at 60 °C. In addition, the time of bleaching was significantly influencing the loss of nitrates.

The modeling results indicated too that the Logarithmic model is the most appropriate (R^2>0.993) model for deducing the transfer energy of the nitrate ions in the food matrix to the bleaching water whatever the treatment temperature.

It would certainly possible to improve the treatment, taking into account other parameters such as: the size of the plant pieces, the solid phase (food matrix / blanching water ratio), and the loss of numerous valuable nutrients (vitamins, some minerals, amino acids, etc.).

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