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Research article

Kinetic modeling of the alkaline deproteinization of Nile-tilapia skin for the production of collagen

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ABSTRACT

A new phenomenological model, based on a second order dissolution kinetics, was developed for the alkaline removal of non-collagenous protein (NCP) from the skin of Nile tilapia (SNT). This model allows estimating the liquid concentration of NCP in terms of temperature, skin size, NaOH concentration and time. This model was fitted with 135 experiments averaging a R^2 of 0.99. The root-mean-square deviation and the mean-absolute-percentage error of the model were 0.0041 and 3.15%, respectively. The Arrhenius-activation energy was 15–122 kJ mol^{-1}. Multi-objective optimization led to the highest NCP extraction (NCPE) of 24.3% and to the lowest loss of collagen (LC) of 1.3%, with R^2 coefficients of 0.98 and 0.92, respectively. Ultimately, SNT deproteinized under optimal conditions was subjected to acid extraction and purification. FTIR and SEM analyses indicated that the product was a Type I collagen that could be used in the pharmaceutical industry.

1. Introduction

Collagen makes up 25–30% of total proteins in animals and it is considered the most abundant protein. Its structure consists of a chain of polypeptides forming a triple helix (Chen et al., 2016). Collagen is present in several tissues of vertebrate animals, mainly as type I, and has been traditionally obtained from their skins and bones for applications in pharmaceuticals, cosmetics, food and nutraceutics (Bhagwat and Dandge, 2016). Health concerns, related to the possible transmission of diseases, and religious restrictions have limited the use of this source of collagen, promoting the search of alternative sources (Chen et al., 2016). Focused has been put on fish residues because they are generated in large quantities worldwide, in spite of this collagen has a lower thermal stability than the mammalian collagen (Blanco et al., 2019; Potaros et al., 2009). In Colombia, 73641 tons of Nile Tilapia (*Oreochromis niloticus*) were produced in 2017, 61% of which were exported as fillet, causing a large generation of byproducts such as the skin (Ministerio de Agricultura, 2017).

The byproducts of Nile Tilapia have been used to obtain collagen under different operating conditions (temperature, extraction time, alkali and acid concentrations and liquid/solid ratio) to evaluate production yield and the thermal stability of the so-obtained collagen (Chen et al., 2016; Li et al., 2018; Potaros et al., 2009). Studies have shown that type I collagen, extracted from the SNT[1], can be used as a biomaterial for tissue regeneration (Li et al., 2018; Song et al., 2006; Zeng et al., 2009; Zhang et al., 2016).

The collagen extraction process comprises two main steps. The first step consists of the removal of NCP[2] and other impurities, such as fat from the skin, aiming to increase the collagen purity. To achieve this, 0.1 M NaOH is generally used, with different stirring times (Kiew et al., 2014; Liu et al., 2015). The second step consists of an acid extraction of the previously NaOH treated skin, commonly using 0.5 M acetic acid (Liu et al., 2015; Wang et al., 2018). After centrifugation, the supernatant containing the acid-soluble collagen is dialyzed and freeze-dried. All procedures are usually done at 4 °C, aiming to preserve the triple helix structure of collagen (Krishnamoorthi et al., 2017; Liu et al., 2014).

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[1] Skin Nile tilapia.

[2] Non-collagenous protein.

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However, Type I collagen is thermally unstable at human body temperature (Leikina et al., 2002) and when the proteins are consumed, they suffer an acid and enzymatic hydrolysis during digestion (Freeman et al., 2004). Therefore, there is no need to carry out the extraction process at these very low temperatures that, besides to the high cooling cost, decrease the extraction rate.

There is abundant information regarding the extraction of collagen from the skin of different marine species (Li et al., 2018), however, there are only few publications dealing with the optimization of the key parameters affecting the extraction process. In particular, most of those publications have studied the parameters temperature, concentrations of NaOH and acetic acid and time (Blanco et al., 2019). Response surface methodology is a statistical tool that has been used for the optimization of collagen-extraction conditions from the fish skin (Blanco et al., 2019). However, none of those studies included all the key optimization parameters influencing the alkaline extraction step, such as skin size and stirring speed (Li et al., 2018; Wang et al., 2018). On the other hand, there are few reports on the kinetic modelling of the acid extraction of collagen from fish byproducts (Kiew et al., 2014) based on physical and empirical methods. For instance, several authors have evaluated empirical models such as power law, parabolic diffusion, Peleg, Elovich and second-order, to describe the acid extraction of collagen from the skin of cultured hybrid catfish (Kiew et al., 2014) and Red Tilapia (Kiew and Mat Don, 2013).

Regarding the alkaline extraction to remove the NCP, some authors have studied the effect of alkaline pretreatment conditions on the collagen-extraction yield and its chemical structure (Liu et al., 2015), in single stage (Sun et al., 2017) and several stages (El-Rashidy et al., 2015). Thus, to the best of our knowledge, this is the first study that models, from a phenomenological point of view, the kinetics of the removal of NCP protein, including experimental factors non-previously studied like skin size and stirring speed. The so-developed model can be a useful tool to facilitate the design, simulation, control and optimization of the process (Bucić-Kojić et al., 2007).

2. Materials and methods

2.1. SNT conditioning

Tilapia was obtained from a local market and it was filleted to remove the skin, which was washed and kept frozen at -20 °C. The SNT was defatted with 10% (v/v) butanol for 16 h at 10 °C, changing the alcoholic solution after 8 h (Nalinanon et al., 2011). The amounts of moisture, fat, ash and total protein of SNT were determined according to the Association of Official Analytical Chemists (AOAC) by the methods 950.46B, 960.39B, 920.153, 981.10, respectively (AOAC, 2003a, 2003b; 2003c, 2003d). The collagen content was determined by hydroxyproline analysis using the Lins Da Silva et al. (2015) protocol. Samples of 100 mg of SNT were used. Absorbance at 560 nm was measured (UV-VIS, Genesys 10S, Thermo scientific) based on a calibration curve obtained with standard hydroxyproline. Collagen content was calculated using a conversion factor of 8 (Blanco et al., 2019; Wang et al., 2018).

2.2. Factorial design and operating conditions for the basic extraction of the NCP

A 3^3 factorial design was used to evaluate the effect of temperature (10–20 °C), NaOH concentration (0.3–0.7 M) and size of SNT (1–5 cm) on the response variables NCPE[3] and the LC[4]. For the alkaline pretreatment, 6 g of defatted SNT was extracted with 200 mL of NaOH solution (Zhong et al., 2014) in a 500 mL jacketed glass reactor stirred at 400 rpm during 5 h (first alkaline extraction). This procedure was repeated twice over 6 h and 7 h (second and third alkaline extraction), in order to eliminate the NCP as much as possible, while keeping minimal collagen losses. The experimental variables were temperature, NaOH concentration and skin size.

The size of skin was included as a design factor because some studies have reported that small changes in size can significantly affect the solid-liquid extraction yield during the washing stage (Qu et al., 2010). Temperature and base concentration ranges were defined based on the reported alkaline pretreatment (Liu et al., 2015) for the production of carp-skin collagen. Extraction times for the first, second and third alkaline treatments were chosen based on the kinetic results observed for these stages in preliminary experiments. The SNT free of NCP, was subjected to continuous washing with distilled water until the pH of residual liquids was neutral (Zhang et al., 2016).

In several studies, the stirring speed value is not reported in any of the stages of the extraction process (Arumugam et al., 2018; Li et al., 2018; Wang et al., 2018), for this reason, some experiments were carried out at different stirring levels fixing the extraction time at low temperature and large size in order to estimate the minimum value of stirring speed, above which, the variation of the percentage of NCP extraction was not significant, and this way, this factor were eliminated from the experimental design.

The volume alkali solution to SNT mass ratio should be high enough that it allows the NCP diffuse properly in the alkaline solvent without increasing the costs of inputs and energy required for extraction (Qu et al., 2010). Thus, an intermediate value for this ratio was defined based on the results obtained in the studies of collagen extraction process from SNT (Potaros et al., 2009; Zeng et al., 2009), to ensure that this ratio did not have a significant effect on the response variables.

2.3. Conditions of acid extraction of collagen

The SNT free of NCP, obtained under optimal conditions, was subjected to extraction with 0.7 M acetic acid (40 mLg^{-1}), stirring at 70 rpm during 5 h ((Kiew and Mat Don, 2012). The extraction was performed at 20 °C because the properties of obtained collagen exhibited non-significant differences with those of the collagen obtained at lower temperatures (Potaros et al., 2009). The solution with the dissolved collagen was subjected to tangential filtration (Vivaflow 200R, in PES de 50 kDa) to eliminate the acetic acid, and it was concentrated by freeze-drying at -40 °C.

2.4. Determination of NCPE and LC

NCPE was estimated using the method of Lowry et al. (1951) at a maximum absorption wavelength of 670 nm in a spectrophotometer (GENESYS 10S UV-Vis, Thermo Scientific, Massachusetts-USA). NCP concentration was estimated using a calibration curve obtained with bovine serum albumin. The NCPE (%) was calculated using Eq. (1) where the NCP content in the SNT was determined according to the Association of Official Analytical Chemists AOAC by the method 981.10 (AOAC, 2003d)

$$\text{NCPE} = \frac{\text{g NCP extrated in the alkaline solution}}{\text{g NCP in SNT}} \times 100 \quad (1)$$

The hydroxyproline content in the basic solution used for deproteinization was estimated following the method of Da Silva et al. (2015). The LC (%) was determined by Eq. (2), from the collagen content in the SNT and in the extracting solution.

$$LC = \frac{g\ collagen\ in\ alkaline\ solution}{g\ collagen\ in\ SNT} \times 100 \quad (2)$$

2.5. Collagen characterization

Morphological characteristics of collagen were determined by SEM analysis (S-4800; Hitachi, Tokyo, Japan) according to Liang et al. (2014).

[3] Non-collagenous protein extraction.
[4] Losses of collagen.

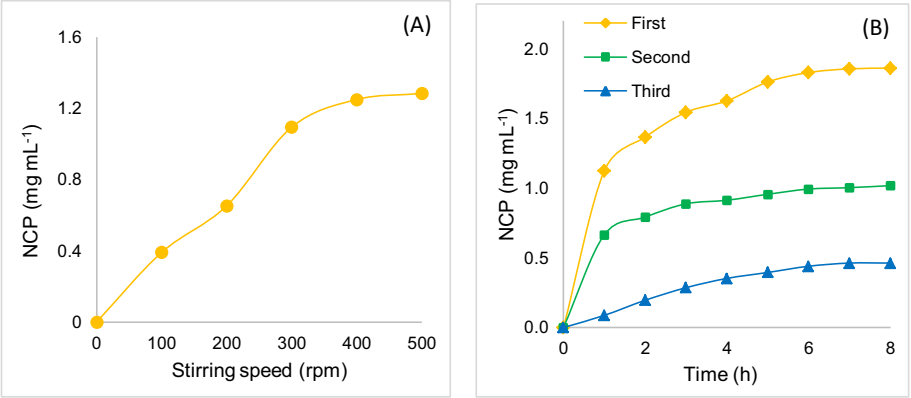

Figure 1. (A) Non-collagen protein (NPC) concentration as function of the stirring speed (Temperature (T) = 10 °C, NaOH concentration (Cb) = 0.5 M, size (S) = 5 cm, time (t) = 5 h). (B) NCP concentration vs. time for first, second and third alkaline extractions (T = 10 °C, Cb = 0.7 M, S = 1 cm).

Freeze-dried collagen was analyzed by infrared spectroscopy (FTIR-ATR, 32 scans, resolution of 4 cm^{-1}), the analysis was performed on a Thermo iS50 device equipped with a diamond crystal (Zhu et al., 2019).

2.6. Kinetic theory

The second order model was evaluated under the following assumptions: a) The pieces of the SNT are isotropic. b) The distribution of the NCP within the SNT is uniform and varies only with respect to time. c) The concentration of NCP is constant at any point of the control volume in a given time. d) The thickness of the SNT is negligible with respect to its length. The following mechanism was proposed for the extraction of the protein (Eq. (3)):

$$Protein(solid) + Solvent \leftrightarrow Soluble\ protein\ (basic\ solution) \tag{3}$$

The model assumes that the dissolution rate depends only on the difference between the concentration of dissolved protein and that in the equilibrium (Zhong et al., 2014).

The differential equation for a second-order kinetics is represented by Eq. (4):

$$\frac{dCt}{dt} = k^*(Ce - Ct)^2 \tag{4}$$

Where k is the second-order extraction rate constant (L g^{-1} h^{-1}), Ce is the concentration of the protein extracted in the equilibrium (g L^{-1}) and Ct is the concentration of non-collagenous protein in the liquid (mg mL^{-1}) at any time t (Qu et al., 2010). Eq. (5) is obtained by substituting the initial conditions ($Ct = 0$ if $t = 0$) in Eq. (4), integrating and making an inversion of the terms.

$$\frac{t}{Ct} = \frac{1}{k^*Ce^2} + \frac{t}{Ce} \tag{5}$$

Coefficients of Eq. (5) were estimated from the slope and the intercept of the graph t/Ct vs. t. The initial extraction rate is defined as h (g L^{-1} h^{-1}) when t and Ct approach 0 through Eq. (6):

$$h = k^*Ce^2 \tag{6}$$

Eq. (5) can be expressed as follows (Eq. (7)):

$$C_t = \frac{t}{\frac{1}{h} + \frac{t}{Ce}} \tag{7}$$

It is possible to find a relationship between k and temperature through the Arrhenius equation (Rakotondramasy-Rabesiaka et al., 2007) which can be expressed as:

$$k = A exp^{\frac{-Ea}{RT}} \tag{8}$$

where A is the pre-exponential factor (L g^{-1} min^{-1}), Ea is the activation energy (J mol^{-1}), R is the ideal gas constant (J mol^{-1} K^{-1}) and T is the absolute temperature (K).

The root mean square deviation (RMSD) and the *P-value* were calculated to estimate the fit quality of the kinetic model (Kiew et al., 2014). A multi-objective optimization was made to obtain the maximum and minimum value for the response variables using a weighting factor of 60% for NCPE and 40% for LC, respectively.

3. Results and discussion

3.1. Proximate composition of SNT

The composition of the wet-based SNT was: 54.91 ± 0.44% moisture, 29.68 ± 0.37% protein (5.01 ± 0.57% collagen), 13.89 ± 0.31% fat and 1.61 ± 0.04% ash. The protein was the solid component in the highest proportion, while collagen content was approximately the 17% of the total protein. This result justifies the elimination of the NCP which corresponded to approximately 25 % of SNT. Akter et al. (2016) reported different results for SNT (71.73% moisture, 21.31% protein, 1.55% fat and 3.88% ash). In addition, Li et al. (2018) found that SNT composition was 64.10% moisture, 21.89% protein, 2.27% fat and 11.7% ash which were also different to our results. These divergences in composition could be attributed to the difference in environmental conditions of the regions where the fish species were obtained or to genetic factors (Villamil et al., 2017).

On other hand, the low ash percentage indicates that the demineralization is not necessary, which is an advantage because this treatment might cause the loosening of the skin matrix (El-Rashidy et al., 2015; Li et al., 2018). The high fat content justifies the defatting stage because fats could affect the filtration process in the membranes and the purity of the final collagen.

3.2. Determination of the conditions for alkaline extraction

The concentration of PNC in the alkali solution does not vary significantly ($p > 0.05$) at stirring speeds above 400 rpm (Figure 1A), therefore, all experiments were performed at that stirring speed, in order to minimize the external mass transfer limitations. Surprisingly, no reports were found on the effect of the stirring speed in any stage of the collagen-extraction process from SNT (Chen et al., 2016; Li et al., 2018; Potaros et al., 2009; Zhang et al., 2016). However, it is possible to compare our results with those obtained in the collagen extraction from Malaysian

Table 1. Response Variables for each treatment of the Factorial Design.

Run	Factors			Response variables	
	T (°C)	Cb (mol L^{-1})	S (cm)	NCPE (%)	LC (%)
1	10.0	0.3	5.0	15.0	0.0
2	10.0	0.3	3.0	16.8	0.0
3	10.0	0.3	1.0	21.8	0.1
4	10.0	0.5	5.0	16.3	0.0
5	10.0	0.5	3.0	17.6	0.0
6	10.0	0.5	1.0	23.4	0.1
7	10.0	0.7	5.0	17.1	0.8
8	10.0	0.7	3.0	18.7	1.2
9	10.0	0.7	1.0	24.3	1.8
10	15.0	0.3	5.0	17.0	0.5
11	15.0	0.3	3.0	18.4	0.1
12	15.0	0.3	1.0	22.9	1.6
13	15.0	0.5	5.0	18.8	1.1
14	15.0	0.5	3.0	19.7	1.0
15	15.0	0.5	1.0	24.3	2.7
16	15.0	0.7	5.0	20.0	2.0
17	15.0	0.7	3.0	21.5	2.6
18	15.0	0.7	1.0	25.5	4.1
19	20.0	0.3	5.0	20.3	2.1
20	20.0	0.3	3.0	20.9	2.3
21	20.0	0.3	1.0	24.1	4.0
22	20.0	0.5	5.0	21.7	3.0
23	20.0	0.5	3.0	22.8	3.2
24	20.0	0.5	1.0	25.6	6.3
25	20.0	0.7	5.0	22.9	3.1
26	20.0	0.7	3.0	24.8	4.2
27	20.0	0.7	1.0	27.2	6.4

T: temperature, Cb: NaOH concentration, S: skin size, NCPE: NCP extraction after 5 h, LC: losses of collagen after 5 h.

cultured Catfish (*Hybrid Clarias Sp.*) (Kiew and Mat Don, 2012), who used the same stirring speed in the acid extraction.

Extraction times for first, second and third alkaline extractions were 5, 6 and 7 h, respectively, based on the initial slope of the curves in Figure 1B. The extraction rate was fast at the beginning of each stage and became slower as the process moved forward.

3.3. Statistical effects of the factors on the response variables

The NCPE and LC results are shown in Table 1, which were fitted with Eqs. (9) and (10), respectively.

$$\sqrt{NCPE} = 4.702 + 0.753Cb + 0.0149T - 0.555S + 0.0107TS + 0.040S^2 \quad (9)$$

$$\sqrt{LC} = 1.895Cb + 0.153T - 0.156S - 1.576 \quad (10)$$

Where *Cb* is NaOH concentration (mol L^{-1}), *T* is temperature (°C) and *S* is size of skin (cm). The R^2 of Eq. (9) was 0.982, indicating a close agreement between the experimental results and values predicted by the statistical model (Kiew and Mat Don, 2012). The error analysis indicated that the coefficient of variation was 1.16%, confirming the validity of the model for the level of confidence employed (Gunst et al., 2006). Eq. (9) exhibited non-significant coefficients for the interaction between the coefficient *T* and *Cb*, *S* and *Cb*, and with the quadratic terms corresponding to T^2 and Cb^2 ($p > 0.05$). According to the p-values, *S* has the main effect on NCPE which is antagonistic, while *Cb* has the lowest but direct effect.

In Eq. (10), only the linear terms had a significant effect ($p < 0.05$) with R^2 of 0.924, which was lower than the obtained for Eq. (9), but still good.

A maximum value of NCPE of 27.2% was obtained for $Cb = 0.7$ M, $T = 20$ °C and $S = 1$ cm, but the maximum value of LC of 6.4% was also obtained at these conditions. For this reason, a multi-objective optimization was performed using the Response Surface Methodology (to analyze the effect of *Cb*, *T* and *S* on NCPE and LC) leading to the optimal conditions 10 °C, 0.7 M NaOH and size of 1 cm, corresponding to maximum value of NCPE of 24.33% and minimum value of LC of 1.76%.

3.4. Kinetic analysis

Figure 2 shows the kinetic plots of the NCP extraction as a function of skin size, temperature and NaOH concentration. It is observed that the skin size has the strongest and inverse effect on the extraction efficiency and this effect is higher at the lower temperatures. This effect of the skin size can be explained because the smaller sizes lead to higher surface areas, allowing a higher protein exposure. Temperature has the second strongest effect, but direct, on the extraction of NCP. However, at the smallest skin size, the effect of temperature becomes weaker. A faster rate was obtained at higher temperatures, indicating a possible Arrhenius behavior. Under the studied levels, NaOH concentration has a mild but direct effect.

In general, all the kinetic plots show two extraction stages. The first stage, that lasts 1–2 h, is the faster one and it is more noticeable at the smaller skins sizes. The second stage is slower and shows an asymptotic trend. It is important to remark that ca. 80% of the total extraction of NPC, which goes up to 5 h, is achieved in the first hour.

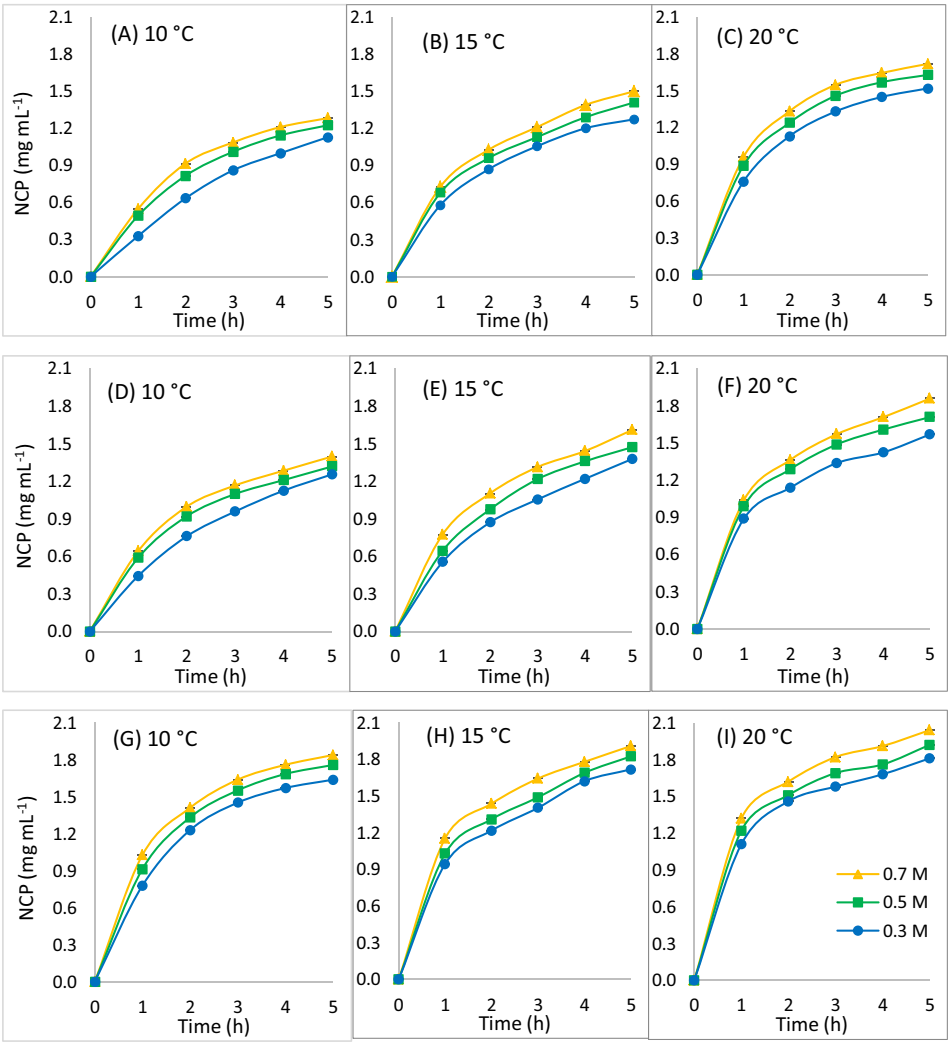

Figure 2. Effects of NaOH concentration (*Cb*), temperature (*T*) and skin size (*S*) on Non-collagen protein (NCP) concentration during the first alkaline extraction. (A), (B), (C) for $S = 5$ cm, (D), (E), (F) for $S = 3$ cm, (G), (H), (I) for $S = 1$ cm. NTS/basic solution = 0.03 and stirring speed of 400 rpm.

All these results strongly suggests that the extraction of NCP indeed behaves as a heterogeneous chemical reaction, which is favored by temperature and by the exhibited surface (Qu et al., 2010; Rakotondramasy-Rabesiaka et al., 2007; Zhong et al., 2014). This fact supports the use of typical reaction kinetics to model the extraction process. In this case, that reaction kinetics approach led to the second order model shown in Eq. (4).

The maximum NCP concentration in the liquid phase was obtained at the smallest skin size (1 cm), the highest temperature (20 °C) and the highest base concentration (0.7 M).

3.4.1. Determination of the empirical parameters of the model

Table 2 shows the obtained empirical parameters (*h*, *Ce* and *k*) of the second-order model. The very high values of R^2 indicated that this model is accurate and, therefore, the equilibrium concentration of NCP (*Ce*) and the extraction rate constant (*k*) can be calculated from the slope and intercept of the straight line based on Eq. (5), respectively. Initial extraction rate (*h*) was estimated from Eq. (6) based on calculated values of *k* and *Ce*.

In this table, it can be seen that the values of the rate constant (*k*) showed a slight increase with *T* but did not show a significant change with *Cb* and *S*. This results are in accordance with those reported by Bucić-Kojić et al. (2007) who concluded that *k* depends exponentially on temperature following the Arrhenius equation. On the contrary, Zhong et al. (2014) did not notice the change of *k* with temperature during the the alkaline extraction of protein from Caragana korshinskii Kom.

Table 2 also shows the Arrhenius parameters (*A* and *Ea*) at different conditions of *Cb* and *S*, which were estimated through the slope and intercept of Arrhenius plot (ln(*k*) vs. *1/T*). *Ea* values were in the range of typical chemical reactions and decreased with an increase in the NaOH concentration, which is a logical variation because at high *Cb* values the protein is released more easily, therefore, the energy required to activate the extraction of NCP from the SNT must be lower. Similarly, when the size of skin is small, the protein is more exposed and the activation energy required is low, which was coherent with the results obtained for this parameter.

Similarly, it is observed that the temperature has a direct effect on the initial extraction rate (*h*) which is consistent with the results reported in the study of antioxidants extraction from pomegranate marc (Qu et al., 2010).

In general, the *Ce* values varied logically with the levels of the experimental factors. The higher *Ce* values were obtained at the higher *Cb* and *T* levels and at the lower levels of S. Results of the effect of *T* on the extraction yields at large time values were also expected because *Ce* is directly related to NCPE (%) (Islam et al., 2013).

Table 2. Parameters of Second Order Model and Arrhenius equation.

Factors			Second-Order Model Parameters				Arrhenius Parameters	
Cb (mol L^{-1})	*S* (cm)	*T* (°C)	Ce (g L^{-1})	*h* (g L^{-1} h^{-1})	*k* (L g^{-1} h^{-1})	R^2	*A* (L g^{-1} h^{-1})	*Ea* (kJ mol^{-1})
0.3	5.0	10	1.75	0.39	0.05	0.98	2.9E+21	122.8
		15	1.84	0.84	0.25	1.00		
		20	2.03	1.26	0.31	1.00		
	3.0	10	2.28	0.56	0.11	1.00	5.8E+15	91.0
		15	2.16	0.73	0.16	1.00		
		20	1.93	1.49	0.40	0.99		
	1.0	10	2.24	1.29	0.26	1.00	1.1E+08	47.0
		15	2.21	1.46	0.30	1.00		
		20	2.11	2.26	0.51	1.00		
0.5	5.0	10	1.94	0.68	0.18	1.00	1.5E+08	48.4
		15	1.94	0.98	0.26	1.00		
		20	2.07	1.58	0.37	1.00		
	3.0	10	1.88	0.87	0.25	1.00	2.3E+05	32. 8
		15	2.19	0.90	0.19	1.00		
		20	2.10	1.76	0.40	1.00		
	1.0	10	2.29	1.57	0.30	1.00	1.1E+06	35.7
		15	2.28	1.64	0.31	1.00		
		20	2.21	2.47	0.50	1.00		
0.7	5.0	10	1.90	0.82	0.23	1.00	9.2E+05	36.0
		15	2.06	1.06	0.25	1.00		
		20	2.15	1.77	0.39	1.00		
	3.0	10	1.94	0.98	0.26	1.00	2.0E+02	15.8
		15	2.18	1.14	0.24	1.00		
		20	2.31	1.74	0.33	1.00		
	1.0	10	2.30	1.86	0.35	1.00	8.5E+03	23.8
		15	2.29	2.08	0.40	1.00		
		20	2.35	2.75	0.50	1.00		

T: Temperature, Cb: NaOH concentration, S: size of skin, Ce: NCP concentration in equilibrium, *h*: initial extraction rate, *k*: second order rate constant. *A*: Pre-exponential factor. *Ea*: Activation Energy.

Table 3. General model validation for some aleatory points.

Factor	*Cb* (mol L^{-1})	*T* (°C)	*S* (cm)	*t* (h)	*Ct*-Experimental (g L^{-1})	*Ct*- Predicted (g L^{-1})	Absolute error (%)
Points into the experimental design	0.4	10	1	4	1.57	1.66	5.96
	0.4	10	5	1	0.49	0.54	7.80
	0.4	10	3	3	1.10	1.16	5.21
	0.4	10	1	5	1.84	1.68	7.20
	0.6	15	5	4	1.20	1.24	2.84
	0.6	15	1	2	1.22	1.30	6.62
	0.6	15	5	5	1.41	1.41	0.13
	0.6	15	1	1	1.16	1.10	7.40
	0.6	20	3	3	1.49	1.49	0.15
	0.6	20	1	2	1.51	1.51	0.24
						Mean absolute error (%)	4.35
Points outside the experimental design	0.4	15	1	1	0.98	0.94	4.20
	0.4	15	1	3	1.44	1.53	6.52
	0.4	15	3	2	0.99	1.00	1.33
	0.6	15	3	4	1.31	1.36	4.39
	0.6	20	1	1	1.25	1.21	3.42
	0.6	20	1	5	1.94	1.94	0.21
	0.73	10	4	2	0.92	0.90	1.89
	0.73	10	4	5	1.32	1.35	2.43
	0.73	18	4	3	1.51	1.37	8.91
	0.73	18	4	4	1.63	1.50	7.59
						Mean absolute error (%)	4.08

Ct is the concentration of non-collagenous protein in the liquid.

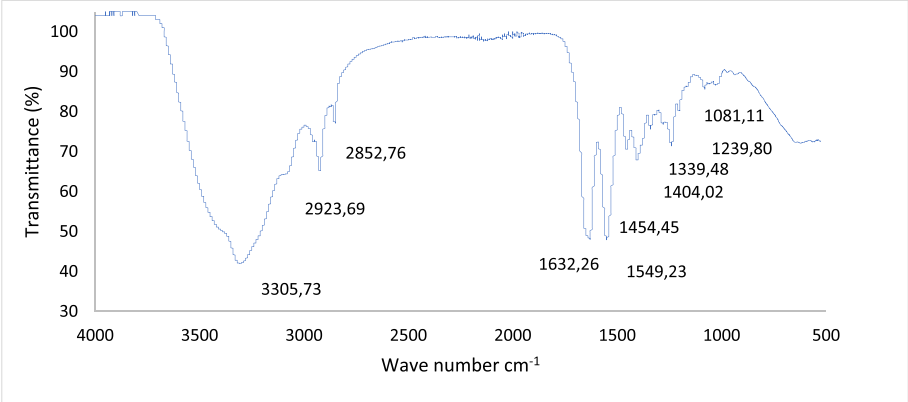

Figure 3. ATR-FTIR analysis of acid-soluble collagen from skin of Nile Tilapia (SNT).

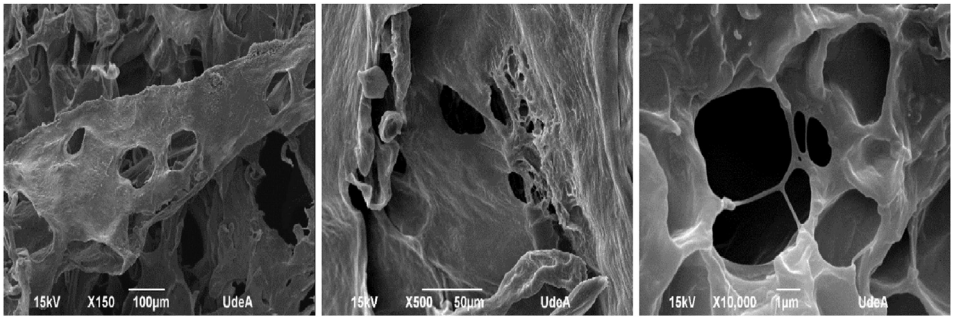

Figure 4. SEM micrographs of extracted collagen from skin of Nile Tilapia (STN).

3.4.2. General model to estimate the NCP concentration

In some previous studies on the kinetics of solid-liquid extractions, correlations of the parameters *Ce*, *h* and *k* were obtained in terms of each of the factors studied in an independent way (Qu et al., 2010; Rakotondramasy-Rabesiaka et al., 2007). However, in this work these correlations were expressed in terms of all factors using solver (Microsoft Excel), in order to determine the effect of each factor on each model parameter, which were shown in Eqs. (11), (12), and (13):

$$Ce = 3.400 - 0.0812T - 2.398Cb - 0.045S + 0.1565TCb \quad R^2 = 0.87 \tag{11}$$

$$k = 0.298 + 0.7036Cb - 0.0278T - 0.1197S - 0.0357TCb + 0.00216T^2 + 0.0146S^2 \quad R^2 = 0.98 \tag{12}$$

$$h = 2.694 - 0.208T + 1.093Cb - 0.755S + 0.0099T^2 + 0.089S^2 \quad R^2 = 0.92 \tag{13}$$

By substituting Eqs. (11) and (13) in the general model (Eq. (7)), it is possible to find a general equation which allows stimating the NCP concentratiion at different values of *t* (h), *Cb* (mol L^{-1}), *T* (°C) and *S* (cm), corresponding to Eq. (14). The root-mean-square deviation (RMSD) and the mean-absolute-percentage error (MAPE) of the model were 0.0042 and 3.15%, respectively. Both prediction-accuracy parameters were calculated from the 135 experimental data for the alkaline pretreatment (Kiew et al., 2014), indicating that the prediction of *Ct* was very good. Table 3 shows the concentrations of non-collagenous protein in the liquid, measured and predicted, as well as the absolute percentage errors for ten aleatory points from the 135 experiments and for ten points outside the experimental design. The mean-absolute-percentage errors of these two sets of experiments were ca. 4.1–4.4%, therefore the model is quite accurate and robust (Kiew et al., 2014).

$$Ct = \frac{t}{\frac{1}{2.69 - 0.21T + 1.1Cb - 0.76S + 0.0099T^2 + 0.089S^2} + \frac{t}{3.40 - 0.081T - 2.4Cb - 0.045S + 0.16TCb}} \tag{14}$$

3.5. Characterization of collagen

Figure 3 shows the ATR-FTIR spectrum of the obtained collagen, with the characteristic peaks (strongest absorbance) in the amides region (A, B, I, II y III). Peaks corresponding to amides A and I were found at 3305 and 1632 cm-1, respectively, while those for amide B, II and III were found in 2923, 1554 cm^{-1} and 1239 cm^{-1}, respectively, which are typical of type I collagen (Chen et al., 2016; Huang et al., 2016; Zhang et al., 2016). The microstructure of the collagen is shown in Figure 4. An irregular filament structure, scratched and not porous, typical of type I collagen is observed (Li et al., 2018).

4. Conclusions

A new phenomenological model, based on a second order dissolution kinetics, was developed for the alkaline removal of non-collagenous protein from the skin of Nile tilapia. This model allows estimating the liquid concentration of the protein in terms of temperature, skin size, NaOH concentration and time. The root-mean-square deviation and the mean-absolute-percentage error of the model, calculated from the 135 experimental data, were 0.0042 and 3.15%, respectively. Besides, the mean-absolute-percentage error for ten points outside the experimental design was 4.1%, therefore, the model is quite accurate and robust. According to the *p-values*, the skin size has the main effect, but inverse, on the extraction percentage of non-collagenous protein, while the NaOH concentration has the lowest but direct effect. The Arrhenius-activation energy was 15–122 kJ mol^{-1}, values in the range of typical chemical reactions, indicating that the extraction was under kinetic regimen. Multi-objective optimization led to the highest extraction percentage of

non-collagenous protein (24.33%) and to the lowest loss of collagen (1.76%), with R^2 coefficients of 0.98 and 0.92, respectively. The phenomenological model has the advantage, respect to mathematical statistical models, of predicting the response variable in a wider range of process conditions. Ultimately, skin of Nile tilapia deproteinized under optimal conditions was subjected to acid extraction and purification. FTIR and SEM analyses indicated that the product was a Type I collagen. The collagen obtained with a yield of 21.4%, which had a purity of 94 %, resulted be a Type I collagen indicating that it can be used in the pharmaceutical industry.

Declarations

Author contribution statement

Diego Enrique Giraldo-Rios: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Luis Alberto Rios & José Edgar Zapata-Montoya: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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