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ARTICLE

# Kinetics and Modeling of Pepsin Soluble Collagen (PSC) Extraction from the Skin of Malaysian catfish (Hybrid *Clarias* sp.)

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**Abstract** The empirical kinetic models for the pepsin soluble collagen extraction from the skin of cultured hybrid catfish (*Claris* sp.) were studied using four two-parametric models, namely the power law, parabolic diffusion, Peleg's and Elovich's models. The Peleg's model was found to be the best model capable of predicting the extraction data with $R^2$ >0.9, *p*-value >2.0%, and RMSD <10.0%, respectively. Kinetic models based on the second order rate equation were successfully developed to describe the extraction processes with different processing variables. Extraction rate constant, initial extraction rate and equilibrium concentrations for different acetic acid concentrations, liquid to solid ratios, and pepsin dosages were predicted. The verification of the developed models showed that the experimental values agreed with the predicted ones, with percentage error differences in the range of 0.03–3.91%.

**Keywords** acetic acid · collagen · diffusion · extraction kinetics · kinetic parameters · modeling

## Introduction

In recent years, collagen has been employed in the food, pharmaceutical, cosmetic, and medical industries (Sadowska et al., 2003). This popular biomaterial is the most abundant animal-derived protein and present in almost all tissues and organs of vertebrates, including the skin, cartilage, bone, muscle, blood vessels as well as various supporting tissues (Yuna et al., 2010). Though collagen can be found in all animal parts, Nakamura et al. (2003) reported that type I collagen was especially concentrated in the skin-associated tissues and bones. Most commercial collagens, however, are derived from bovine hide, pig skin, and chicken wastes, which created much anxiety among health-conscious consumers for the past decades due to the outbreaks of bovine spongiform encephalopathy, foot-and-mouth disease, and avian flu (Ali, 2010). In addition, pessimism and strong concerns persist with regard to the usage of land animal-derived collagens, particularly due to religious sentiments as collagens extracted from bovine sources are prohibited for Sikhs and Hindus, whilst porcine collagen cannot be consumed by Muslims and Jews (Kiew and Mashitah, 2012a). Consequently, alternative sources, mainly those of aquatic sources have been paid increasing attention serving as the potential replacement for collagens of mammalian and land animals.

Numerous researches were intensified on the functional properties of aquatic collagens, especially extraction from fish skin, because they represented important sources of highly soluble collagens (Giménez et al., 2005). These include skin of yellowfin tuna (Woo et al., 2008), skin of striped catfish (Singh et al., 2011), skins of young and adult Nile perch (Muyonga et al., 2004), skin of grass carp (Wang et al., 2009), skin of Baltic cod (Sadowska et al., 2003), skin of bigeye snapper (Kittiphattanabawon et al., 2005), skin of brown backed toadfish (Senaratne et al., 2006), and skin of skate (Hwang et al., 2007). Fish collagen was therefore undoubtedly able to serve as a viable alternative for safer and consumer-friendly collagen. In our preliminary study, cultured hybrid catfish of *Clarias* sp. (*Clarias gariepinus* × *C. macrocephalus*) was found to contain the highest amount of pepsin-soluble collagen (PSC), preceding other Malaysian freshwater fishes such as red tilapia (*Oreochromis niloticus*), black tilapia (*Oreochromis mossambicus*), pangasius catfish (*Pangasius sutchi*), Sultan fish (*Leptobarbus hoevenii*), and labyrinth fish (*Trichogaster trichopterus*) (Kiew and Mashitah, 2012b). Locally known as *Keli*, this hybrid catfish has been a staple freshwater fish in

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Malaysia as a good source of protein with a considerable amount of collagen in the muscles and skins (Sivakumar et al., 2000). Nevertheless, commercial value of this hybrid *Clarias* sp. was much lower as compared to other cultured fishes due to its abundancy in the market. Anon (2011) reported that cultured catfish production in Malaysia showed significant improvement in recent years by 7-folds from 7, 158 tons in 1999 to 81,041 tons in 2009. Hence, attempts have been made to boost up the commercial value of this cultured catfish by utilizing the skin as the raw material for collagen extraction.

Collagen extraction is a solid-liquid extraction process, also known as leaching, through which collagen is obtained from raw materials (i.e. fish skin) through dissolution with a suitable solvent. The simplified mechanism of collagen mass transfer involves two stages, namely rapid washing of collagen molecules from the superficial sites of fish skin surfaces, and slow diffusion of collagen molecules located at the internal sites through the skin particles and from the skin into the bulk of the liquid extract. According to Kitanoviæ et al. (2008), external resistance to mass transfer was usually assumed to be insignificant; therefore, diffusion of collagen through fish skin particles was the rate limiting step of the overall extraction process in their study. Based on the mechanism of mass transfer assumed, the mathematical model of extraction kinetics was then defined. In engineering point of view, kinetic model is a useful tool, which considerably facilitates process optimization, simulation, design, and control (Buciæ-Kojiæ et al., 2007; Kitanoviæ et al., 2008). Additionally, mathematical modeling of collagen extraction processes is also important in order to reduce energy, time, and chemical reagent consumption (Piwowarska and González-Alvarez, 2012). Therefore, in the development of collagen isolation process, the extraction kinetics of collagen from the skins of hybrid *Clarias* sp. is essential for reactor design and process optimization. Despite many studies reported on the possibility of various raw materials as potential alternatives for mammalian collagen, a suitable kinetic model for collagen extraction is not yet available in the literature. The present study, therefore, aimed at proposing an appropriate model to describe the extraction kinetics of collagen from the skins of hybrid *Clarias* sp. Four empirical models, namely the power law, parabolic diffusion, Peleg's and Elovich's models were compared to each other. Our main goal was to choose the optimum empirical kinetic model based on its accuracy of fitting the experimental data obtained at different operating conditions. In addition, the influences of processing parameters, including acid concentration (A), liquid to solid ratio (R), and pepsin dosage (E), on the extraction yield were investigated. The kinetic models of extraction processes under different parameters were then established to predict the extraction yield and reveal the extraction mechanism.

## Materials and Methods

**Materials.** Cultured catfish (hybrid of *C. gariepinus* × *C. macrocephalus*) were purchased from a local wet market in Parit Buntar, Perak, Malaysia. Upon arrival at the laboratory, the fishes were killed, dissected, and deboned. The adhered tissues on the skins were cleaned before being cut into small pieces (1 cm × 1 cm). The skin were then washed with distilled water and kept frozen at −20°C prior to collagen extraction.

**Chemicals.** Commercial pepsin from porcine gastric mucosa, sodium hydroxide, and acetic acid were purchased from Merck Sdn. Bhd. (Malaysia). All other chemicals used were of analytical grade.

**Extraction of pepsin soluble collagen (PSC).** All procedures were performed as described by Wang et al. (2009) and Kittiphattanabawon et al. (2005), with slight modifications. The extraction process was carried out at 4°C. To remove non-collagenous proteins, the skins were mixed with 0.1 M NaOH at a sample to alkali ratio of 1:20 (w/v). The mixture was stirred for 6 h. The NaOH solution was changed every 2 h. The sample was then washed thoroughly with excessive distilled water until the pH was neutral or slightly basic. Deproteinised skins were defatted with 10% butyl alcohol with a sample to alcohol ratio of 1:20 (w/v) for 24 h. The alcohol solution was changed at 8-h intervals. Defatted skins were then washed with cold water and subjected to collagen extraction using aqueous acetic acid. They were actively stirred in acetic acid with varying concentrations (0.1, 0.3, 0.5, 0.7, and 0.9 M) and acetic liquid to skins ratios (10, 20, 30, 40, and 50 mL/g) containing pepsin (0, 0.5, 1, 1.5, 2, and 2.5%; w/w) for 24 h, in order to extract pepsin soluble collagen. The viscous collagenous material was separated from the insoluble components by high speed centrifugation at 20,000×g for 40 min, and the soluble collagen solution was obtained from the supernatant. The collagen was precipitated by adding NaCl so as to obtain a final concentration of 0.8 M. Resulting sediment was collected by centrifugation at 20,000×g for 30 min. To further purify the collagen, it was re-dissolved in minimal amount of acetic acid, dialyzed against 0.1 M acetic acid, followed by distilled water and lyophilized. The freeze-dried product was designated as PSC. The wet yield of PSC from the skin of *Clarias* sp. was calculated using Eq. (1):

$$\text{Yield of collagen (wet) (\%)} = \frac{\text{Weight of collagen (g)}}{\text{Weight of wet skin (g)}} \times 100$$

## Proposed Empirical Kinetic Models

Numerous researches have been conducted to describe the kinetics

and mechanism of extraction process, particularly solid liquid extraction for various vegetal (plant) tissues (Sturzoiu et al., 2011). However, there is no kinetic model for collagen extraction from fish skins reported so far. Kinetic models can be divided into physical and empirical models. According to Kitanoviæ et al. (2008), empirical models described the mathematic variations of the amount of extracted compound in either the raw material or liquid extract with time. They were normally simpler as compared to the physical models but were appropriate for engineering purposes. In the present study, four two-parametric kinetic models namely power law, parabolic diffusion, Peleg's and Elovich's equations, which were commonly applied in the modeling of solute recovered from different types of solid materials (plant materials, soil, ores, and wastes) were proposed to represent the kinetics of collagen extraction based on the following assumptions:

- fish skins were isotropic and of equal size;
- distribution of collagen within the fish skins was uniform and varied only with time;
- net diffusion occurred only towards the external surface of fish skins; and
- diffusion coefficient of collagen was a constant.

**Power law model.** The power law model, which was similar to Freundlich type, was applied widely in the diffusion process of an active agent through non-swelling devices (Sturzoiu et al, 2011). It could be applied as follows:

$$y = Bt^n \tag{2}$$

where $y$ is the yield of collagen (g/g), $B$ refers to the constant incorporating the characteristics of the carrier-active agent system, $t$ is the time in minutes, and $n$ is the diffusional exponent, an indicative of transport mechanism. In literature, $n$ was less than 1 for extraction from plant or vegetal materials. The constants for this model must be estimated using a regression analysis. In the linearized form, the equation was transformed into:

$$ln\ y = n\ ln\ t + ln\ B \tag{3}$$

**Parabolic diffusion model.** Orthogonal polynomial was another useful empirical equation in solid liquid extraction (Kitanoviæ et al., 2008). With the general form:

$$Y(x) = \Sigma_{i=0}^{n} A_i \varnothing_i(x) \tag{4}$$

where $A_i$ is the parameter to be determined and $\varnothing_i$ is the function of $x$. Therefore, the yield of collagen, $y$, was given by:

$$y = \Sigma_{i=0}^{n} A_i t^{1/2} \tag{5}$$

Kim et al. (2002) fitted the third-degree polynomial in the form of Eq (6) to the leaching data of the simulated and real paraffin waste.

$$y = A_0 + A_1 t^{1/2} + A_2 t \tag{6}$$

The three terms in Eq (6) represented three kinetic behaviors observed during leaching. The first term was accounted for the washing of loosely bound materials, which would be leached instantaneously, whereas the second and third represented diffusive release and chemical reactions (dissolution, corrosion, or solubility control), respectively (Kitanoviæ et al., 2008). In the present study of collagen extraction, it was assumed that there was no chemical reaction involved. Eq (6) could be simplified into:

$$y = A_0 + A_1 t^{1/2} \tag{7}$$

This was known as the parabolic diffusion equation, which corresponded to two-step extraction mechanisms, consisting of washing, followed by diffusion. $A_0$ is known as the washing coefficient, indicating the amount of collagen extracted instantaneously when the fish skins were submerged into acetic acid (solvent). Likewise, $A_1$ is known as the diffusion rate constant.

**Peleg's model.** As confirmed by this experimental work, shape of the collagen extraction curves was similar to that of sorption curves (moisture content versus time). Therefore, it is possible to describe these collagen extraction curves using the model proposed by Peleg (Buciæ-Kojiæ et al., 2007) which is also known as the hyperbolic model. In the case of extraction, the model is adapted and used in the form of Eq (8):

$$y = y_0 + \frac{t}{K_1 + K_2 t} \tag{8}$$

where $y_0$ is the initial yield of collagen extracted at $t = 0$, $K_1$ is the Peleg's rate constant (min g skins/g collagen) and $K_2$ is the Peleg's capacity constant (g skins/g collagen). Since $y_0$ for all fish skins in extraction process is zero, Eq (8) is simplified and used in the form:

$$y = \frac{t}{K_1 + K_2 t} \tag{9}$$

**Elovich's equation.** The following logarithmic relation was a modified form of Elovich's equation. It was fitted to leaching curves such as the extraction of polycyclic aromatic hydrocarbons from coal tar-contaminated soil (Paterson et al., 1999). The relationship assumed that the rate of adsorption could be replaced by the rate of extraction, and leaching rate decreased exponentially with increasing extraction yield. It was expressed as in Eq (10):

$$y = e\ ln\ t + a \tag{10}$$

**Second order rate law.** Extraction of natural ingredients and kinetic model building of many extraction processes have been reported in the literature based on the assumption of mechanism of a second order leaching. Charpe and Rathod (2012) described that the mechanism of the second order kinetic model was suitable to govern solid-liquid extraction processes. The general second order kinetic model can be written as:

$$\frac{dC_t}{dt}=k(C_e-C_t)^2 \tag{11}$$

where $k$ is the second-order extraction rate constant (mL/mg h), $C_e$ is the equilibrium concentration of total collagen in the liquid extract (mg/mL), and $C_t$ is the total collagen concentration in the liquid extract at a given extraction time t (mg/mL).

**Kinetics of extraction.** Experimental results of collagen extraction were analyzed using the linearized equations of the proposed empirical kinetic models. Model parameters were calculated by linear regression using Microsoft Excel software.

**Model building of collagen extraction process.** Similar approach as proposed by Charpe and Rathod (2012) and Qu et al. (2010) was applied in the present study to develop a model which could predict the collagen extraction rate constant, initial collagen extraction rate, and equilibrium concentration. The integrated rate law for the second order extraction (Eq. 11) can be obtained by using boundary conditions of $t$=0 to t, and $C_t$=0 to $C_t$. The integration and rearrangement of the equation are shown below:

$$\frac{dC_t}{dt}=k(C_e-C_t)^2 \tag{11}$$

$$\int_0^{C_t}\frac{1}{(C_e-C_t)^2}dC_t=k\int_0^t dt \tag{12}$$

$$\left[\frac{1}{C_e-C_t}\right]_0^{C_t}=k[t]_0^t \tag{13}$$

$$\left[\frac{1}{C_e-C_t}\right]-\left[\frac{1}{C_e}\right]=kt-k(0) \tag{14}$$

$$\frac{C_e-C_e+C_t}{C_e(C_e-C_t)}=kt \tag{15}$$

$$\frac{C_t}{C_e^2-C_eC_t}=kt \tag{16}$$

$$C_t=kt(C_e^2-C_eC_t) \tag{17}$$

$$C_t=ktC_e^2-ktC_eC_t \tag{18}$$

$$C_t+ktC_eC_t=ktC_e^2 \tag{19}$$

$$C_t(1+kt)=ktC_e^2 \tag{20}$$

Therefore, the integrated equation can be written as:

$$C_t=\frac{C_e^2kt}{1+ktC_e} \tag{21}$$

And after linearization Eq. (21) can be presented as:

$$\frac{t}{C_t}=\frac{1}{kC_e^2}+\frac{t}{C_e} \tag{22}$$

Then when t approaches 0, the initial collagen extraction rate, h (mg/mL. h), can be written as:

$$h=kC_e^2 \tag{23}$$

After rearranging Eq. (21) and (23), $C_t$ can therefore be expressed as:

$$C_t=\frac{t}{\left(\frac{1}{h}\right)+\left(\frac{t}{C_e}\right)} \tag{24}$$

The initial collagen extraction rate, $h$ (mg/mL h), the second order extraction rate constant, $k$ (mL/mg h), and the equilibrium concentration of total collagen in the liquid extract, $C_e$ (mg/mL) were determined experimentally from the slope and intercept of the graph of $t/C_t$ versus t. It was assumed that the second order kinetic model could be applied to evaluate the influences of the process variables: acid concentration (A), liquid to solid ratio (R), and pepsin dosage (E) on the extraction efficiency. Thus, the $h$, $C_e$, and $k$ were related to these variables and the kinetic model for the prediction of collagen yield at different process variables was then obtained.

**Statistical methods.** In order to validate the models, the kinetic parameter values obtained from collagen extraction were used to simulate the profiles for the proposed two-parametric kinetic models. The profiles from simulation of the experimental data and models were then evaluated using the linear correlation coefficient, $R^2$, and the root mean square deviation (RMSD) computed as follow (Buciæ-Kojiæ et al., 2007; Jokiæ et al., 2010):

$$RMSD=\sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_{exp}-y_{calc})^2} \tag{25}$$

Another criterion used to evaluate the best fitting equation was the mean relative percentage deviation ($p$) value. $p$ value is defined as:

$$P=\frac{100}{N}\times\sum\frac{|Y-Y_p|}{Y} \tag{26}$$

where $Y$ and $Y_p$ are experimental and predicted yield of collagen, respectively, and $N$ is the number of experimental data. A model is considered acceptable if the $p$ values are below 10% (Figen and Gedik, 2004).

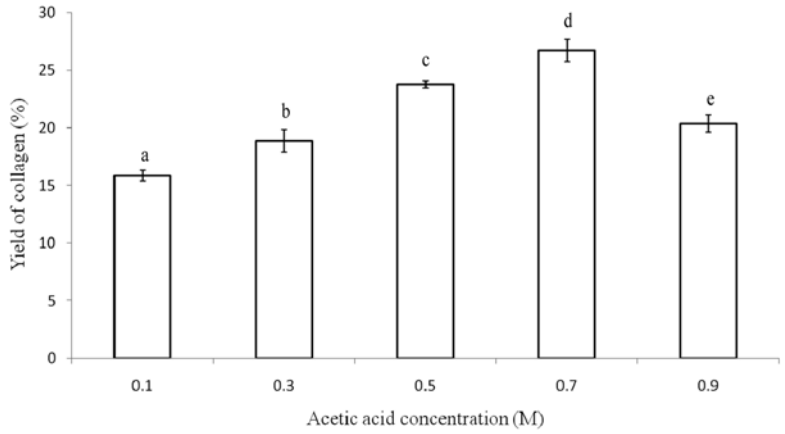


**Fig. 1** Yield of collagen at different concentrations of acetic acid as the extracting medium. The liquid to solid ratio and pepsin dosage were set at 30 mL/g and 1.5% (w/w), respectively. The column with the same alphabet letter was not significantly different ($p$ >0.05).

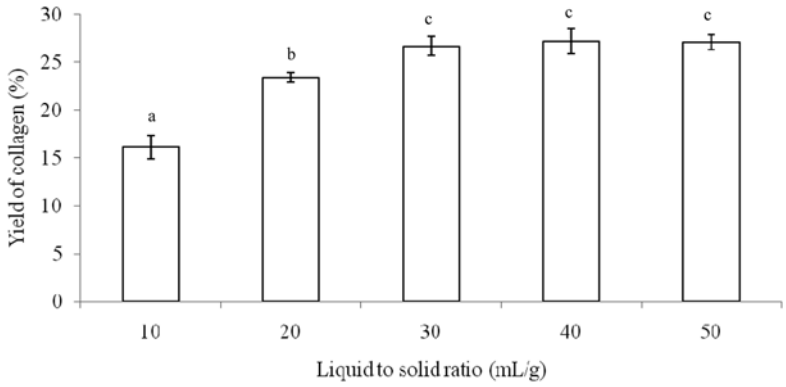


**Fig. 2** Yield of collagen at different liquid to solid ratio. The acetic acid concentration and pepsin dosage were set at 0.7 M and 1.5% (w/w), respectively. The column with the same alphabet letter was not significantly different ($p$ >0.05).

## Results and Discussion

**Effect of acetic acid concentration.** Acetic acid was the most favourable extracting solvent used in collagen extraction studies from marine and land animals. Few researchers particularly Cheng et al. (2009) and Skierka and Sadowska (2007) stated that the extraction of collagen from animal tissues through acetic acid showed better efficiency and higher yield than other organic acids as well as inorganic acid (e.g. hydrochloric acid). Fig. 1 shows the effect of different concentration of acetic acid on the yield of PSC from the skin of hybrid *Clarias* sp. Yield of PSC increased with the increased acetic acid concentration to 0.7 M. However, a reverse trend was observed beyond this concentration. The highest yield was achieved when 0.7 M acetic acid was used as the extracting medium and PSC as much as 26.69±0.97% was extracted. For higher concentration of acetic acid particularly at 0.9 M, the yield of PSC was 20.35±0.75%, which was significantly ($p$ <0.05) lower than that of 0.7 M.

Among the concentrations of acid tested, 0.1 M was the least effective concentration for PSC extraction from the skins. Incomplete solubility of the skins suggested that inter-molecular cross-links were still present in collagen molecules. According to Skierka and Sadowska (2007), the initial stage of collagen solubilisation was the hydration of fibrous collagen, which was proceeded by exposure to acids. As the concentration of acetic acid increased, the modification of electrostatic interaction and structure of the collagen occurred due to the changes in pH (Verheul et al., 1998). In the present study, extracting medium of very low pH (0.9 M, pH 2.39) reduced the collagen water absorption ability. Skierka and Sadowska (2007) mentioned that the positively charged amine groups of proteins formed bonding with anions at very low pH (refers to $CH_3COO^-$ for acetic acid aqueous solution), hence leading to weaker electrostatic repulsive forces between the one-nominal charged group. This resulted in tightening of the structure of collagen fibres and reduced the ability to form bonding with water, thus decreasing the solubility of collagen in the medium. In addition, Wang et al. (2009) also pointed out that collagen was denatured at extremely low pH value as collagenous fibres start to shrink at pH around or below 2.0, making protein hydration impossible. These explained the

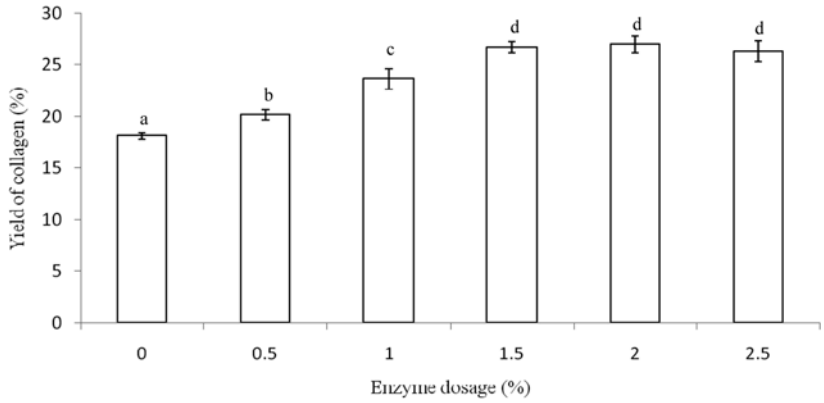


**Fig. 3** Yield of collagen at different pepsin dosages. The acetic acid concentration and liquid to solid ratio were set at 0.7 M and 30 mL/g, respectively. The column with the same alphabet letter was not significantly different ($p$> 0.05).

observations in our study that beyond the acetic acid concentration of 0.7 M, a significant decrease in the yield of PSC was obtained.

**Effect of liquid to solid ratio.** Liquid to solid ratio is an important variable affecting the efficiency of extraction. In the present study, the effect of the amount of solvent (acetic acid) to *Clarias* sp. skin ratio on the extractability is shown in Fig. 2. An increasing liquid to solid ratio could lead to higher yield of PSC. The ratio varied from 10–50 mL/g; however, when it was raised to more than 30 mL/g, improvement in the yield of PSC was no longer significant. Wang et al. (2009) reported that at higher liquid to solid ratio, the concentration gradient and diffusion rate of collagen particles from the fish skins into acetic acid increased, thereby enhancing the efficiency of extraction process. Nevertheless, using a large amount of solvent is not cost-effective due to higher operating cost of solvent and waste handling at the end of the extraction process. Consequently, the ratio of acetic acid to the skins ratio of 30 mL/g was appropriate for PSC extraction carried out in our study.

**Effect of pepsin dosage.** Acid extraction with pepsin digestion is a common method for collagen extraction nowadays. As described by Skierka and Sadowska (2007), the enzyme (pepsin) removed only the non-helical ends of the collagen. Not only the physico-chemical properties of collagen were altered, but non-collagenous proteins were also hydrolyzed. Therefore, it is possible to expect an increase in the collagen solubility after enzymatic treatment. In the present study, the influence of pepsin addition into the extracting medium was obvious, in which the yield of collagen increased significantly from 18.11±0.32% to more than 20% at the end of the extraction period. As presented in Fig. 3, hybrid *Clarias* sp. skin was not completely solubilized by 0.7 M acetic acid, since the lowest yield of collagen was observed when pepsin was not added. The extracted collagen under this condition was designated as acid-soluble collagen (ASC), in agreement with Singh et al. (2011) and Skierka and Sadowska (2007), who reported that incomplete solubilization of striped catfish skin and trash fish in 0.5 M acetic acid, without pepsin digestion. Enzymatic treatment of fish skins with pepsin assisted in removing only the non-helical ends (telopeptides) of the collagen, which act as the inter-molecular crosslink (Skierka and Sadowska, 2007). The difference in yields of ASC and PSC from the skin of *Clarias* sp. might be due to the existence of inter-molecular crosslink at the telopeptide regions of the collagen that resulted in lower solubility in acid. Following pepsin digestion, cross-linked regions at the telopeptides were cleaved without damaging the integrity of the triple helix. This led to higher solubility of collagen in acid (Zhang et al., 2007). Such increase in collagen solubility, regardless of the sources after pepsin treatment, was also reported by other authors (Bama et al., 2010; Huang et al., 2011). A drastic increase in the yield of collagen was obtained when the pepsin dosage was increased up to 1.5% (w/w) (Fig. 3). This observation was as expected, because the higher the amount of pepsin added into the extracting medium, more telopeptides were cleaved, and solubility of collagen particles into the medium was improved. However, further increment of pepsin dosage higher than 1.5% (w/w) no longer resulted in significant improvement of the yield ($p$ >0.05).

**Comparison of empirical models.** Washing was characterized by the rapid increase in the yield of collagen in the beginning of the extraction process, followed by slow extraction approximately after the first 16 h of the process. Fig. 4–7 illustrate the profiles of experimental and simulated data for the extraction of collagen from the skin of *Clarias* sp. at different process conditions using the power law, parabolic diffusion, Peleg's, and Elovich's models. The predicted results were in relatively good agreement to the experimental data, with the linear correlation coefficient, ($R^2$) values above 0.85. In fact, the $R^2$ values were high for all the empirical models, ranging $0.854<R^2<0.996$ (Table 1A–D). This showed that the proposed empirical models were sufficient to describe both the fast washing action and slow-diffusion of collagen extraction process under different process conditions (Kiew and Mashitah, 2012a). The $R^2$ value is frequently used to judge whether the model correctly represents the data, implying that, if $R^2$ is close to one, then the regression model is correct

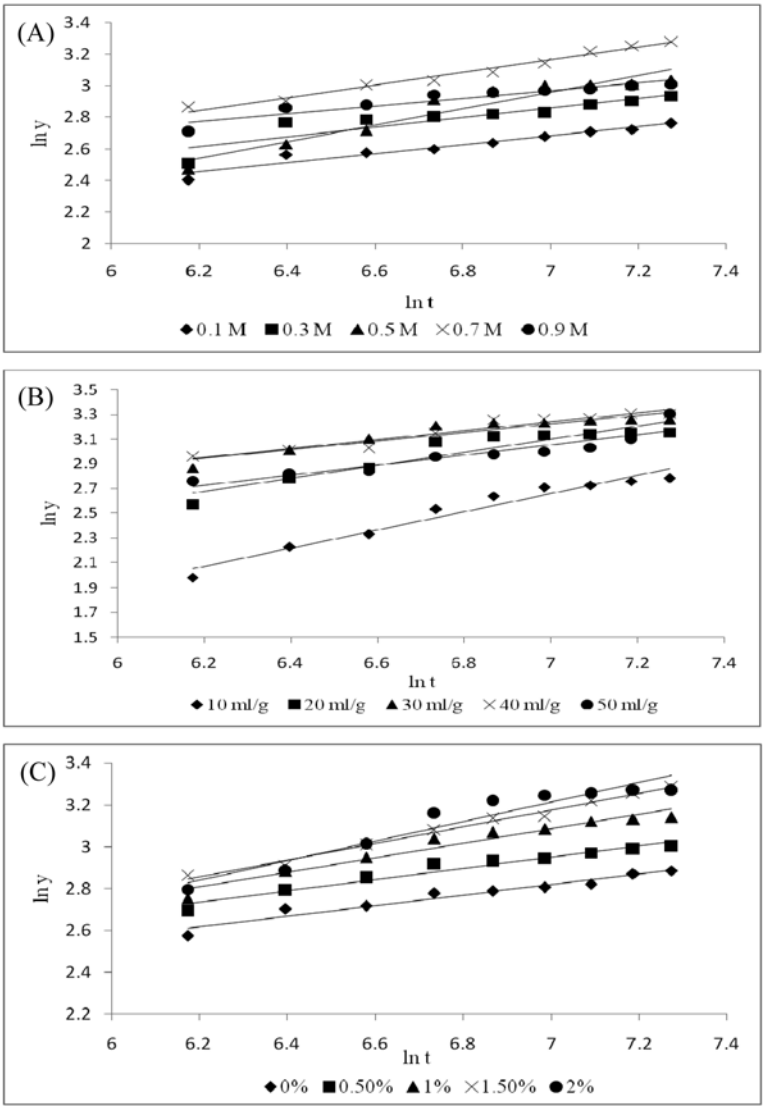


**Fig. 4** Comparison of experimental (symbols) and fitted (line) data for the extraction of collagen from *Clarias* sp. based on linearized form of kinetic equation of the power law model under different process conditions: (A) Acetic acid concentration (B) Liquid to solid ratio (C) Pepsin dosage.

(Annuar et al., 2008). However, many examples exist where the $R^2$ is closed enough to one but the model is still not appropriate. Hence the RMSD was used with the $R^2$ for the comparison of various empirical extraction models. A model with small RMSD represents the data more accurately than the models with larger RMSD (Kitanoviæ et al., 2008).

Table 1 (A–D) shows the calculated parameters for the proposed empirical models and the corresponding statistical correlation values, indicating that regardless of the process conditions, individual average value of the RMSD was lower than 10% for the Peleg's model, in contrast to all other models. In order to further evaluate the best fitting model in predicting the yield of skin collagen from the extraction processes, and $p$ (%) values corresponding to each proposed empirical model were calculated. Even though a model is acceptable if the $p$ values are below 10% as suggested by Figen and Gedik (2004), the Peleg's model gave the lowest $p$ values, which were not higher than 2% for all process conditions and less than 10% for the RMSD, making it the most satisfactory model in representing the processes. In other words, correlation between the amounts of collagen extracted by pepsin digestion from the skins of *Clarias* sp. related to time could be appropriately represented by the Peleg's equation, suggesting that a diffusive mechanism governed collagen release from the fish skins. Kitanoviæ et al. (2008) mentioned that the Peleg's model was derived from the second order rate law which was successfully applied to model solid liquid extraction in various studies. Therefore, it was stipulated that the second order kinetic model could be applied to evaluate the effects of the process variables,

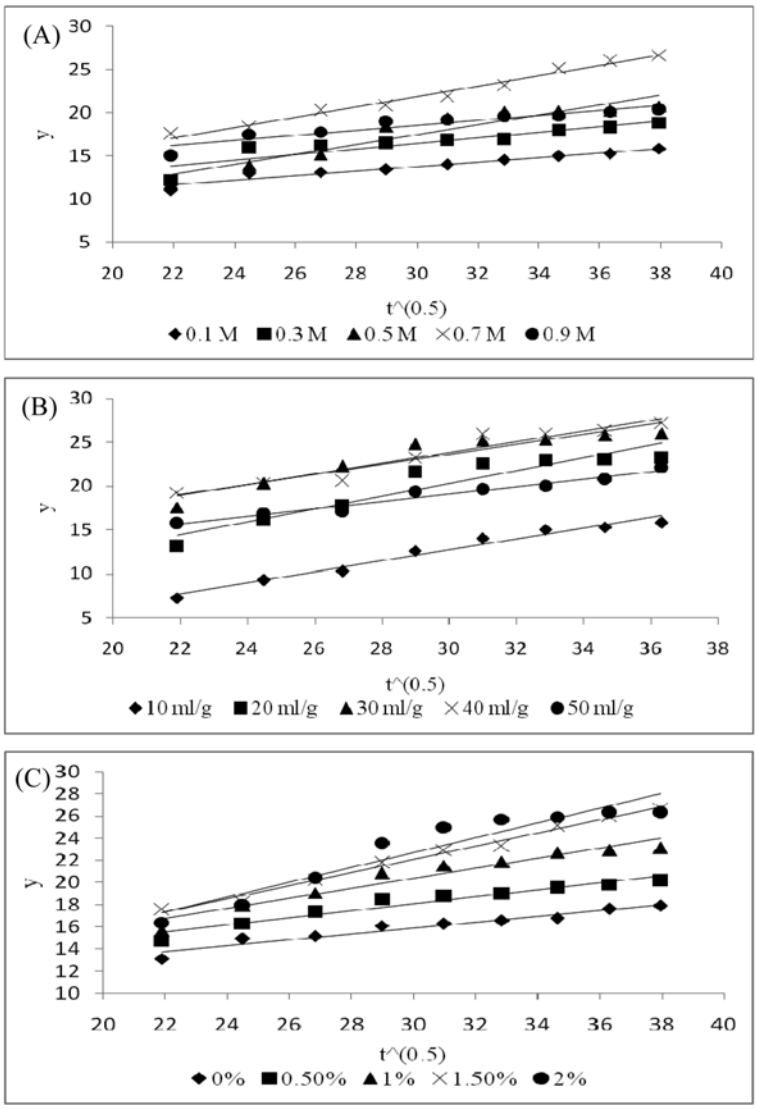


**Fig. 5** Comparison of experimental (symbols) and fitted (line) data for the extraction of collagen from *Clarias* sp. based on linearized form of kinetic equation of the parabolic diffusion model under different process conditions: (A) Acetic acid concentration (B) Liquid to solid ratio (C) Pepsin dosage.

including acid concentration (A), liquid to solid ratio (R), and pepsin dosage (E) on the extraction efficiency, thus the kinetic model for the prediction of extraction yield of collagen for different process conditions could be obtained.

**Developed collagen extraction kinetic model .** The graphs of $t/C_t$ and $t$ for different acetic acid concentration, liquid to solid ratio, and pepsin dosage were plotted, and the values of extraction rate constant, $k$, initial extraction rate, $h$, and equilibrium concentration, $C_e$, were obtained from the slope and intercept of these graphs (Table 2). Subsequently, the kinetic model for the prediction of extraction yield of collagen for different acetic acid concentrations, liquid to solid ratio, and pepsin dosages was obtained.

Kinetic parameters increased with the increase of acetic acid concentration up to 0.7 M, as expected based on the experimental results. This was similar to the yield profiles (Fig. 1). Because the $k$, $h$, and $C_e$ were dependent on acetic acid concentration (A), values for different A values were nonlinearly fitted by polynomial functions with high correlation coefficient ($R^2$=1). These functions are expressed as:

$$C_{e(A)} = -177.6A^4 + 321.8A^3 - 196.3A^2 + 50.17A + 4.217 \ (R^2=1) \quad (27)$$

$$h_{(A)} = -52.93A^4 + 100.2A^3 - 61.75A^2 + 14.99A - 0.29 \ (R^2=1) \quad (28)$$

$$k_{(A)} = 0.062A^3 - 0.06A^2 + 0.015A + 0.011 \ (R^2=1) \quad (29)$$

Therefore, $C_t$ based upon acetic acid concentration is obtained by substituting the above equations in Eq. (24) as:

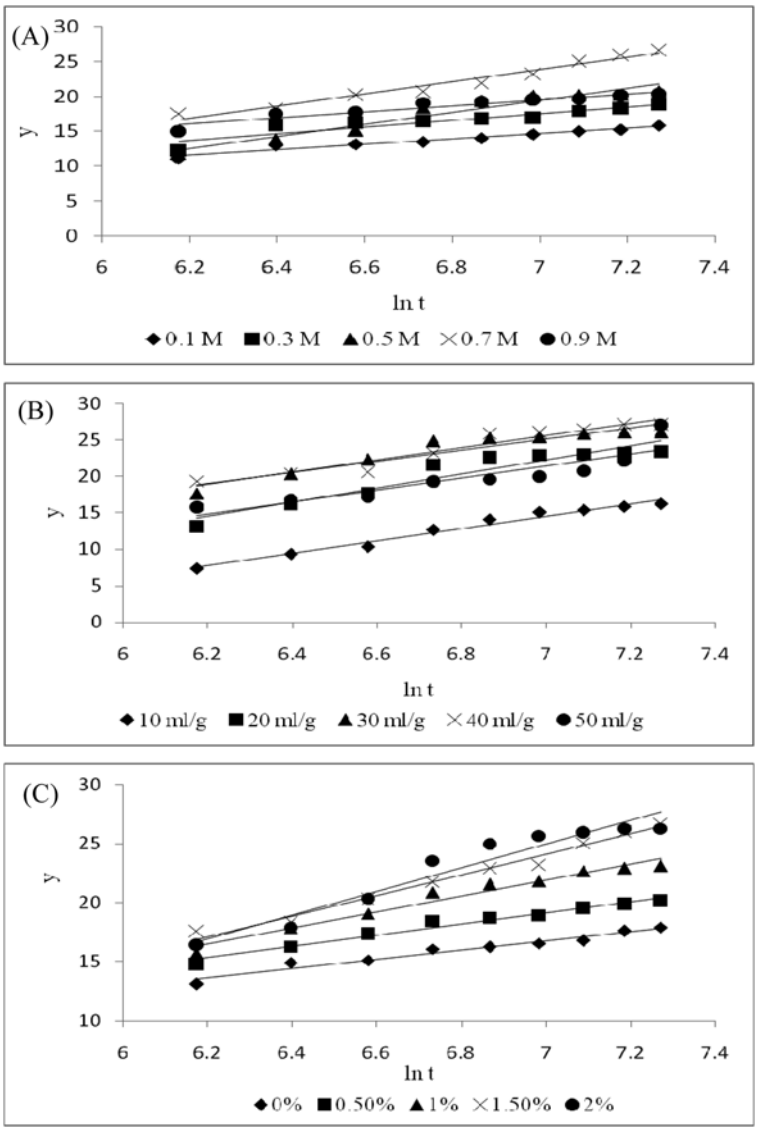


**Fig. 6** Comparison of experimental (symbols) and fitted (line) data for the extraction of collagen from *Clarias* sp. based on linearized form of kinetic equation of the Elovich's model under different process conditions: (A) Acetic acid concentration (B) Liquid to solid ratio (C) Pepsin dosage.

$$C_{t,A} = t \Big/ \left( \frac{1}{-52.93A^4 + 100.2A^3 - 61.75A^2 + 14.99A - 0.29} + \frac{t}{-177.6A^4 + 321.8A^3 - 196.3A^2 + 50.17A + 4.217} \right) \quad (30)$$

This equation can be applied to predict the yield or concentration of collagens extracted from skins of hybrid *Clarias* sp. under different concentrations of acetic acid at a given time with the liquid to solid ratio of 30 mL/g and pepsin dosage of 1.5% (w/w). Collagen extraction at liquid to solid ratio (R) of 10 mL/g resulted in the highest $h$ and $C_e$ values compared to those at ratios of 20, 30, 40, and 50 mL/g. This was due to the highest amount of fish skin present in the solvent. Nevertheless a reverse trend was observed for the $k$ value. The highest $k$ value was achieved at ratio of 50 mL/g, followed by 40, 30, 20, and 10 mL/g. This was due to the fact that higher liquid to solid ratio increased the concentration gradient and enhanced diffusion rate of collagen particles from the fish skins into acetic acid. According to the model assumption, the parameters ($h$, $k$, and $C_e$) were expressed by the variable of R, and they were written in the form of equations as below:

$$C_{e(R)} = -12.1 lnR + 52.68 \ (R^2 = 0.996) \quad (31)$$

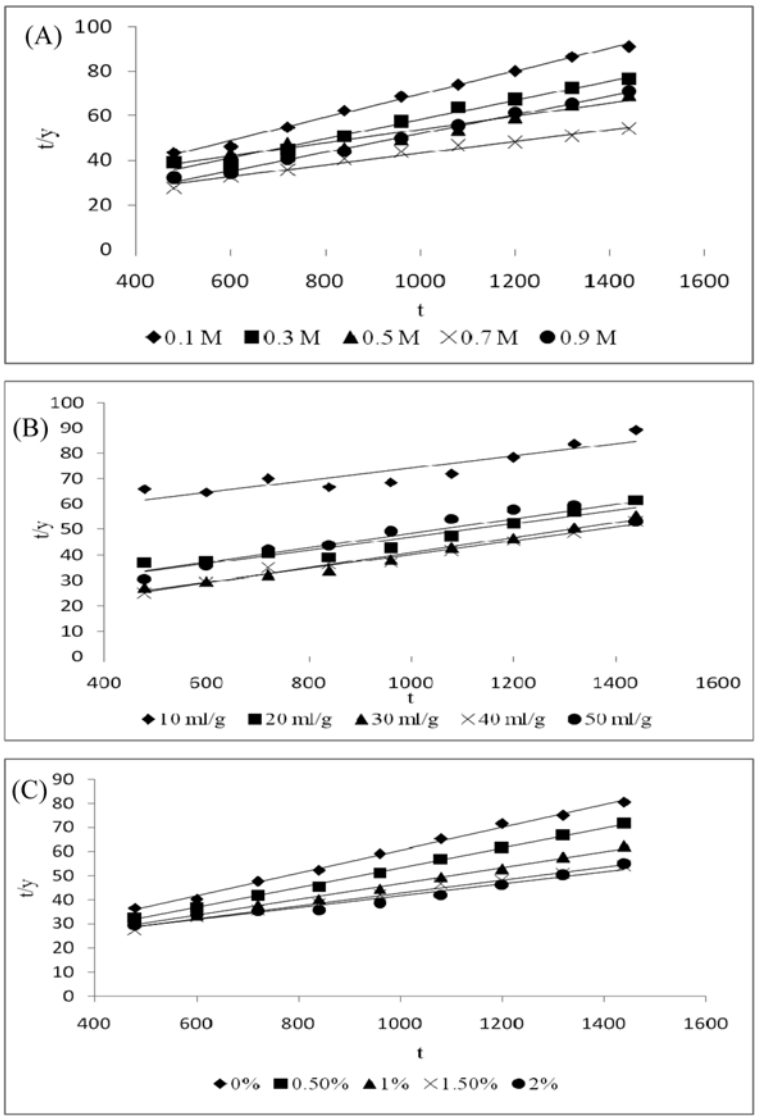


**Fig. 7** Comparison of experimental (symbols) and fitted (line) data for the extraction of collagen from *Clarias* sp. based on linearized form of kinetic equation of the Peleg's model under different process conditions: (A) Acetic acid concentration (B) Liquid to solid ratio (C) Pepsin dosage.

$$h_{(R)} = (-7\times10^{-5})R^3 + 0.005R^2 - 0.139R + 2.547 \quad (R^2 = 0.999) \tag{32}$$

$$k_{(R)} = (2\times10^{-5})R^2 + 0.002 \quad (R^2 = 0.999) \tag{33}$$

Substituting the $h_{(R)}$ and $C_{e(R)}$ into Eq. (24), the relationship can therefore described as:

$$C_{t,R} = \frac{t}{\dfrac{1}{(-7\times10^{-5})R^3+0.005R^2-0.139R+2.547} + \dfrac{t}{-12.1lnR+52.68}} \tag{34}$$

This equation can be used to predict the yield or concentration of collagen extracted from skins of hybrid *Clarias* sp. under different liquid to solid ratios at a given time with acetic acid concentration at 0.7 M and pepsin dosage of 1.5% (w/w).

Similar analysis was undertaken for the effect of variation of pepsin dosage (E). As mentioned earlier, improvement in the collagen yield was obtained when higher amount of pepsin was added into the extracting medium. More telopeptides were cleaved, enhancing solubility of collagen molecules into acetic acid. This was in accordance with the values of $C_e$ calculated, which increased with increasing dosage of pepsin. Highest $C_e$ was achieved with 2% pepsin. Hence, the variations of kinetic

**Table 1** Summary of model parameters and statistical correlation values for each empirical kinetic model at different process conditions: (A) Power law (B) Parabolic diffusion (C) Elovich's model (D) Peleg's model

(A)

| | Empirical Model (Parameters) | | Statistical Correlation Values | | |
|---|---|---|---|---|---|
| | Power Law | | $R^2$ | RMSD (%) | $P$ (%) |
| | n | $B$ ($min^{-n}$) | | | |
| Acid Concentration (M) | | | | | |
| 0.1 | 0.783 | 0.064 | 0.910 | 17.785 | 1.533 |
| 0.3 | 0.703 | 0.133 | 0.904 | 16.523 | 0.791 |
| 0.5 | 0.678 | 0.172 | 0.970 | 8.534 | 0.271 |
| 0.7 | 0.599 | 0.372 | 0.937 | 11.215 | 0.259 |
| 0.9 | 0.581 | 0.349 | 0.907 | 13.470 | 0.413 |
| Liquid to Solid Ratio (mL/g) | | | | | |
| 10 | 0.783 | 0.059 | 0.982 | 7.664 | 0.155 |
| 20 | 0.598 | 0.428 | 0.976 | 6.405 | 0.060 |
| 30 | 0.564 | 0.401 | 0.903 | 14.101 | 0.570 |
| 40 | 0.543 | 0.626 | 0.854 | 16.039 | 0.632 |
| 50 | 0.536 | 0.481 | 0.967 | 7.227 | 0.023 |
| Pepsin Dosage (%) | | | | | |
| 0.0 | 0.266 | 2.601 | 0.967 | 2.128 | 0.058 |
| 0.5 | 0.349 | 1.672 | 0.957 | 3.426 | 0.341 |
| 1.0 | 0.439 | 1.013 | 0.965 | 4.653 | 0.324 |
| 1.5 | 0.466 | 0.926 | 0.973 | 3.246 | 0.368 |
| 2.0 | 0.508 | 0.710 | 0.97 | 4.838 | 0.295 |

(B)

| | Empirical Model (Parameters) | | Statistical Correlation Values | | |
|---|---|---|---|---|---|
| | Parabolic Diffusion | | $R^2$ | RMSD (%) | $P$ (%) |
| | $A_0$ | $A_1$ ($min^{-0.5}$) | | | |
| Acid Concentration (M) | | | | | |
| 0.1 | 0.513 | 1.914 | 0.895 | 22.278 | 5.906 |
| 0.3 | 0.591 | 2.229 | 0.893 | 21.149 | 4.813 |
| 0.5 | 0.663 | 2.596 | 0.964 | 8.575 | 1.605 |
| 0.7 | 0.736 | 0.416 | 0.966 | 15.151 | 2.547 |
| 0.9 | 0.571 | 0.777 | 0.891 | 15.307 | 8.093 |
| Liquid to Solid Ratio (mL/g) | | | | | |
| 10 | 0.563 | 0.431 | 0.977 | 7.949 | 0.632 |
| 20 | 0.605 | 0.623 | 0.957 | 6.709 | 0.605 |
| 30 | 0.645 | 1.299 | 0.918 | 6.527 | 4.066 |
| 40 | 0.659 | 2.569 | 0.904 | 6.970 | 4.330 |
| 50 | 0.699 | 4.424 | 0.948 | 6.924 | 0.937 |
| Pepsin Dosage (%) | | | | | |
| 0.0 | 0.284 | 7.358 | 0.956 | 2.494 | 0.092 |
| 0.5 | 0.352 | 7.284 | 0.947 | 2.982 | 0.017 |
| 1.0 | 0.530 | 4.428 | 0.930 | 5.118 | 0.333 |
| 1.5 | 0.602 | 3.990 | 0.992 | 1.527 | 0.060 |
| 2.0 | 0.690 | 1.888 | 0.934 | 4.665 | 0.242 |

parameters with E were represented by third order polynomial functions as:

$$C_{e(E)} = -2.351E^3 + 7.328E^2 - 2.17E + 7.066 \ (R^2 = 0.988) \quad (35)$$

$$h_{(E)} = 0.367E^3 - 1.27E^2 + 0.87E + 1.551 \ (R^2 = 0.977) \quad (36)$$

$$k_{(E)} = 0.009E^3 - 0.027E^2 + 0.003E + 0.031 \ (R^2 = 0.999) \quad (37)$$

Substituting these equations into Eq. (24) gave:

**Table 1** Continued

(C)

| | Empirical Model (Parameters) | | Statistical Correlation Values | | |
|---|---|---|---|---|---|
| | Elovich's model | | $R^2$ | RMSD (%) | $P$ (%) |
| | e | a | | | |
| Acid Concentration (M) | | | | | |
| 0.1 | 5.945 | 26.880 | 0.936 | 18.411 | 1.083 |
| 0.3 | 6.838 | 30.250 | 0.932 | 22.001 | 2.014 |
| 0.5 | 7.532 | 33.490 | 0.970 | 12.690 | 0.752 |
| 0.7 | 8.446 | 35.250 | 0.991 | 2.865 | 0.132 |
| 0.9 | 6.713 | 27.270 | 0.958 | 7.645 | 1.107 |
| Liquid to Solid Ratio (mL/g) | | | | | |
| 10 | 5.945 | 26.880 | 0.931 | 14.542 | 1.635 |
| 20 | 6.838 | 30.250 | 0.943 | 8.934 | 0.203 |
| 30 | 7.532 | 33.490 | 0.978 | 4.122 | 1.052 |
| 40 | 8.446 | 35.250 | 0.959 | 6.051 | 1.941 |
| 50 | 8.713 | 27.270 | 0.939 | 6.650 | 0.173 |
| Pepsin Dosage (%) | | | | | |
| 0.0 | 6.236 | 24.260 | 0.939 | 8.187 | 2.571 |
| 0.5 | 6.459 | 28.000 | 0.972 | 3.754 | 0.969 |
| 1.0 | 7.374 | 29.680 | 0.990 | 3.278 | 0.320 |
| 1.5 | 8.556 | 35.800 | 0.995 | 2.351 | 0.257 |
| 2.0 | 9.171 | 39.360 | 0.983 | 4.324 | 0.640 |

(D)

| | Empirical Model (Parameters) | | Statistical Correlation Values | | |
|---|---|---|---|---|---|
| | Peleg's model | | $R^2$ | RMSD (%) | $P$ (%) |
| | $K_1$ | $K_2$ | | | |
| Acid Concentration (M) | | | | | |
| 0.1 | 17.720 | 0.051 | 0.994 | 2.426 | 1.002 |
| 0.3 | 15.210 | 0.043 | 0.980 | 4.522 | 0.093 |
| 0.5 | 14.330 | 0.029 | 0.939 | 4.662 | 0.769 |
| 0.7 | 16.620 | 0.026 | 0.980 | 3.446 | 1.013 |
| 0.9 | 10.270 | 0.041 | 0.996 | 2.665 | 1.501 |
| Liquid to Solid Ratio (mL/g) | | | | | |
| 10 | 50.900 | 0.023 | 0.981 | 9.488 | 0.077 |
| 20 | 19.090 | 0.027 | 0.950 | 6.808 | 0.835 |
| 30 | 14.660 | 0.026 | 0.958 | 5.060 | 0.434 |
| 40 | 12.840 | 0.027 | 0.959 | 4.743 | 0.674 |
| 50 | 18.440 | 0.029 | 0.939 | 6.625 | 0.762 |
| Pepsin Dosage (%) | | | | | |
| 0.0 | 16.140 | 0.047 | 0.996 | 2.018 | 0.946 |
| 0.5 | 15.020 | 0.041 | 0.998 | 1.126 | 0.181 |
| 1.0 | 14.940 | 0.031 | 0.992 | 3.093 | 1.841 |
| 1.5 | 12.830 | 0.027 | 0.990 | 3.138 | 1.356 |
| 2.0 | 12.150 | 0.025 | 0.972 | 3.901 | 0.897 |

$$C_{t,E}=\frac{t}{\frac{1}{0.367E^3-1.27E^2+0.87E+1.551}+\frac{t}{-2.35E^3+7.328E^2-2.17E+7.066}} \tag{38}$$

**Table 2** Extraction rate constant, initial extraction rate and equilibrium concentration for different extraction process conditions

| Process Variables | Values | Initial extraction rate, $h$ (mg/mL.hr) | Equilibrium concentration of collagen, $C_e$ (mg/mL) | Extraction rate constant, $k$ (mL/mg. h) |
|---|---|---|---|---|
| Acetic acid concentration (M) | 0.1 | 0.686 | 7.576 | 0.012 |
| | 0.3 | 0.928 | 8.850 | 0.012 |
| | 0.5 | 0.991 | 9.346 | 0.013 |
| | 0.7 | 1.623 | 10.870 | 0.014 |
| | 0.9 | 1.538 | 8.403 | 0.022 |
| Liquid to solid ratio (mL/g) | 10 | 1.661 | 25.000 | 0.003 |
| | 20 | 1.492 | 15.625 | 0.006 |
| | 30 | 1.623 | 10.870 | 0.014 |
| | 40 | 1.608 | 8.130 | 0.024 |
| | 50 | 1.034 | 5.155 | 0.039 |
| Pepsin dosage (%) | 0.0 | 1.560 | 6.993 | 0.032 |
| | 0.5 | 1.481 | 7.8125 | 0.028 |
| | 1.0 | 1.370 | 9.434 | 0.018 |
| | 1.5 | 1.203 | 12.658 | 0.008 |
| | 2.0 | 1.156 | 13.157 | 0.007 |

**Table 3** Experimental verification of concentration of extracted collagen $C_t$ with model prediction at different extraction time (t), acetic acid concentration (A), liquid to solid ratio (R), and pepsin dosage (E)

| | | Predictive (mg/mL)* | Experimental (mg/mL)** | Error (%)*** |
|---|---|---|---|---|
| $C_{t,A}$ | $C_{5h,\ 0.9M}$ | 3.973 | 3.923 | 1.285 |
| | $C_{9h,\ 0.9M}$ | 5.194 | 5.401 | 3.833 |
| | $C_{13h,\ 0.9M}$ | 5.891 | 5.943 | 0.875 |
| | $C_{17h,\ 0.9M}$ | 6.341 | 6.480 | 2.145 |
| | $C_{21h,\ 0.9M}$ | 6.656 | 6.658 | 0.030 |
| $C_{t,R}$ | $C_{3h,\ 40mL/g}$ | 2.234 | 2.325 | 3.914 |
| | $C_{7h,\ 40mL/g}$ | 4.080 | 4.218 | 3.272 |
| | $C_{11h,\ 40mL/g}$ | 5.268 | 5.145 | 2.391 |
| | $C_{15h,\ 40mL/g}$ | 6.097 | 5.996 | 1.684 |
| | $C_{23h,\ 40mL/g}$ | 7.176 | 6.985 | 2.734 |
| $C_{t,E}$ | $C_{3h,\ 0.5\%}$ | 3.054 | 2.949 | 3.561 |
| | $C_{9h,\ 0.5\%}$ | 5.056 | 5.192 | 2.619 |
| | $C_{11h,\ 0.5\%}$ | 5.376 | 5.475 | 1.808 |
| | $C_{17h,\ 0.5\%}$ | 5.977 | 6.144 | 2.718 |
| | $C_{21h,\ 0.5\%}$ | 6.220 | 6.125 | 1.551 |

*Predictive is the predictive value of concentration of extracted skin collagen by using respective mathematical model of each process variable.
**Experimental is the experimental value of concentration of extracted skin collagen.
***Error is the difference between experimental and predicted values and is expressed as percentage (%) of experimental value.

Yield or concentration of skin collagen from hybrid *Clarias* sp. can therefore be predicted using Eq. (38) for different pepsin dosage when acetic acid concentration is at 0.7 M and liquid to solid ratio of 30 mL/g.

**Validation of developed model.** Table 3 shows the predictive and experimental concentrations of skin collagen from hybrid *Clarias* sp. under the extraction conditions that were different from the ones used for the model development. The satisfactorily fitted experimental data with low errors ranging from 0.030–3.914% were obtained. This indicated that the developed models can be used for predicting the extraction performances.

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