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Alkaline Separation of Protein from Canola Meal and Its Kinetic Study

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Abstract

Canola protein is an abundant crop product which finds application as a component in several industrial bioproducts. Knowledge of the effect of separation parameters and limiting factors of protein separation is valuable in scale-up and in optimizing protein separation. The effect of separation parameters (sodium hydroxide concentration: 0.02M to 0.08M; particle size: 16 mesh to 120 mesh; temperature: 25°C to 55°C) on the separation rate and protein yield were studied by kinetic analysis using a two-site model and Peleg's model. Protein yield and separation rate increased with increasing alkaline concentration, increasing temperature and decreasing particle size. Particle size had the greatest effect on equilibrium yield with a 269.68mg·g^{-1} increase in yield between particle ranges of 16-35 mesh and 60-120 mesh. The maximum equilibrium protein yield (779.90mg·g^{-1}) was obtained at a particle size of 60-120 mesh and 0.06M sodium hydroxide concentration at 25ºC. The initial separation rate was accelerated at higher temperatures (2858.42mg·g^{-1}·s^{-1} at 55°C).

Keywords: Canola protein; Alkaline separation; Kinetics; Two-site model; Peleg's model

Introduction

Canola is an oilseed crop which ranks second in the world after soybean, for the production of edible oil bearing seed [1–4], with an average production of 71 million metric tons per year [5]. Canola seed is primarily used to produce canola oil, which constitutes 40% of the seed [2,6]. The remaining 60% of the seed comprises a protein rich meal which remains as an underutilized by-product after oil extraction. Currently, the average global production of canola meal is at 39 million metric tons, which is 13% of the major oilseed meal production around the world [5]. Owing to the growing demand for canola oil in the food and biodiesel industries [7], there is expected to be a rise in global canola meal production [5]. Since canola meal constitutes approximately 60% of the oilseed [8], its valorization plays an important role in maintaining the profitability of canola seed processing operations [6,9,10].

Canola meal contains 20% to 50% protein on a dry basis, which is similar to the protein content of soybean meal [1,11,12]. However, while soybean meal is used extensively in the food industry, the utilization of canola meal has been confined primarily to animal feeds or fertilizers (Canola Council 2009; Li et al. 2017; Gerzhova et al. 2015a). Potential applications of the protein component in canola meal have been explored by researchers in human food consumption [6,13–15], industrial products such as adhesives [16], plastics [17], bio-composites, and other environmentally friendly products [7,18]. Such applications typically require the protein to be separated from the solid meal matrix [19,20]. Alkaline separation followed by acid precipitation is a commonly used technique to obtain plant protein [1–3,10,15,21–23]. This technique is also used commercially in the separation of soybean protein [10] and is a preferred method as it solubilizes hydrophobic proteins more effectively than other methods [24].

Alkaline separation works on the principle that proteins attain a net negative or net positive charge when adjusted to alkaline or acidic pH conditions due to the respective loss or gain of protons. Like charges cause strong repulsion and consequent solubilization of the protein at these extreme pH conditions [20,25]. Generally pH values of 10 to 12 have been used for alkaline protein dissolution [1,2]. At a certain pH, both negative and positive charges on the protein are balanced and the net charge on the protein is zero. At this point, repulsive electrostatic forces are reduced, and protein aggregation occurs because of attractive forces between the protein molecules. The pH at which this phenomenon occurs, is called the isoelectric point, pI of the protein [26]. The pI value of canola protein has been found to be within the range of 3.5 to 5.5 [1,2,21]. Alkaline separation is

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a two-step process which involves (1) separation of the protein from the solid matrix by solubilization in a high alkaline pH liquid solution and (2) precipitation at the isoelectric point by a pH change using acid [9,10,14,24]. The first step, protein dissolution is thought to be the rate limiting step as factors such as concentration of alkaline, pH of the solution, particle size and temperature play a role in protein solid-liquid mass transfer [24]. Understanding the effect of the extraction conditions on the rate and yield of protein extraction is useful in determining limiting factors and conditions favoring a profitable operation for commercialization and scale up. The objective of this work was therefore, to study the effects of the separation conditions (alkaline concentration, particle size and temperature) on the yield and separation rate of protein from canola meal. Sodium hydroxide was used to carry out alkaline separation at various conditions. Two kinetic models (two-site model and Peleg's model) were assumed, to study the effects of the separation conditions on the initial rate and final yield of the separation.

Materials and Methods

Canola meal defatting

Expeller pressed canola meal (35.6% protein, Carbon Cycle Crush LLC, Oroville, WA, USA) was sieved (<16 mesh) and dried in an oven at 49°C for 18h. The meal was defatted three times by stirring in fresh n-hexane (99%, Alfa Aesar, Ward Hill, MA, USA) at a meal to hexane ratio of 1:3 (w/v) for 1.5 h [1,15,27]. The meal was filtered and dried in a fume hood for 24h to remove residual hexane, separated by sieving into particle ranges of 16-35 mesh (1.19mm–0.5mm), 35-60 mesh (0.5mm–0.25mm) and 60-120 mesh (0.25mm–0.125mm) and stored in an airtight bag at -20°C until further use.

Protein assay standard preparation

To determine the amount of protein separated at various conditions, a standard defatted protein isolate with known protein content was prepared from canola meal. Protein was separated from 25g of expeller pressed canola meal (<16 mesh, Carbon Cycle Crush LLC, Oroville, WA, USA) by stirring with 250mL of 0.1M sodium hydroxide for 2h [1,10,27]. The mixture was centrifuged at 3000g for 20min [10,15] and the supernatant was separated by vacuum filtration. The pH of the supernatant was adjusted to 4, using 5M hydrochloric acid (Alfa Aesar, Ward Hill, MA, USA) and stirred for 15min to allow aggregation of protein followed by centrifugation at 3000g for 20min. The resultant protein isolate was washed twice with 30mL of distilled water, with centrifugation of 3000g for 10min between each wash. The protein isolate was freeze dried. The protein and fat content of the isolate were determined at Central Analytical Laboratory (University of Arkansas, Fayetteville, AR) by combustion and ether separation methods respectively. The isolate was defatted at a meal to solvent ratio of 1:12 for 1.5h, as described above, and stored at -20°C for further use.

Alkaline separation of protein

5g of defatted canola meal was stirred in 125mL of sodium hydroxide (Fisher BioReagents, Fair Lawn, NJ, USA) of various concentrations [24] at 200rpm for 1800s [10]. Aliquots of 1 mL were taken at 10s intervals for the first 120s, followed by 20s to 30s intervals thereafter. The samples were immediately added into 49mL of distilled water (to dilute and lower the pH of the sample and to prevent further protein separation) and centrifuged at 3000g for 20min at 4°C (Jouan C4i Centrifuge, Thermo Electron Corporation). A pH meter (Fisher Science Education) was used to monitor the pH of the separation

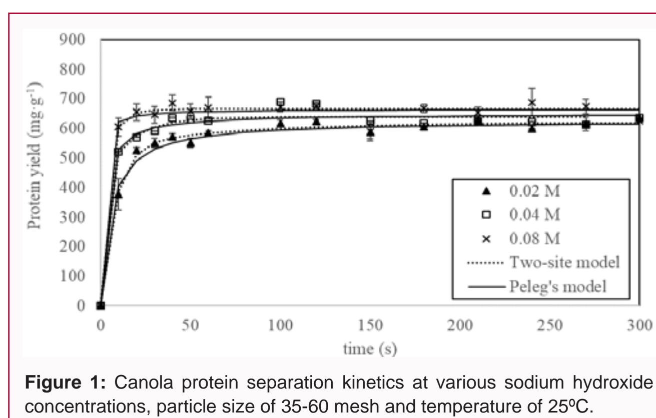

Figure 1: Canola protein separation kinetics at various sodium hydroxide concentrations, particle size of 35-60 mesh and temperature of 25ºC.

mixture. Separations at temperatures higher than room temperature, were conducted in a water bath. The sodium hydroxide solution was heated to the required temperature in the water bath prior to the separation and the meal was heated to the required temperature in an oven. The temperature of the separation solution was controlled by a thermocouple attached to the heating source.

Protein determination

The bicinchoninic acid (BCA) protein assay (Pierce BCA reagent kit, Thermo Scientific, Rockford, IL, USA) was used for protein determination of the aliquot samples. Canola protein of a known purity was used as standard for the protein determination (see Section 2.2). The samples and standards were incubated for 30min at 37ºC after adding the BCA reagent and the absorbance was determined at 562nm at room temperature using a microplate reader (BioTek, PowerWave HT).

Kinetic Models

Two-site model

Plant meal consists of classes of particles which release protein at different rates. Broken particles constitute the fraction of meal which releases protein at a fast rate and the remaining fraction which constitutes intact cells, release the protein at a slower rate. In the two-site model, two parallel processes of reaction are considered, which occur at faster and slower rates respectively. The two-site model can be described by two first order expressions as follows [24,28–30]:

$$C_t = C_1\left(1-e^{-k_1 t}\right)+C_2\left(1-e^{-k_2 t}\right) \quad (1)$$

where C_t is the yield of protein in the solution (mg·g^{-1}) at separation time, t(s), C_1 and C_2 are the protein yields (mg·g-1) from the fast and slow processes respectively, and k_1 and k_2 are the first order rate constants describing release of protein from the fast and slow processes respectively (s^{-1}). The total yield of protein in the solution at equilibrium, C_{eq} (mg·g^{-1}) can be expressed as follows:

$$C_1+C_2=C_{eq} \quad (2)$$

The values of c_1, c_2, k_1 and k_2 were obtained by fitting the experimental data to the two-site model (Equation 1) by non-linear regression using the software, QtiPlot 5.6.1.

Peleg's model

Peleg's model is a semi-empirical model developed for the description of sorption isotherms of food materials [31]. Extraction curves hold a similar shape to sorption curves and therefore Peleg's model has been adapted widely by researchers for kinetic analysis of solid-liquid separation from plant materials, with some modification.

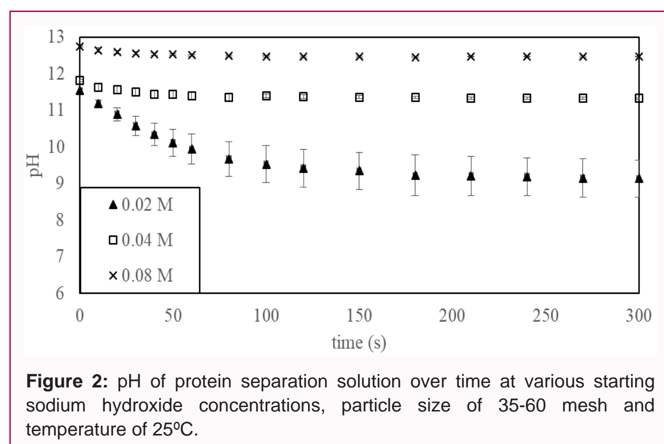

Figure 2: pH of protein separation solution over time at various starting sodium hydroxide concentrations, particle size of 35-60 mesh and temperature of 25ºC.

Peleg's model entails two stages of reaction, namely a first order reaction in the initial stage, followed by a zero-order separation in the latter stage. The Peleg's model, modified for separation with fresh solvent, is given below (Equation 3) [28,32–34]:

$$C_t = \frac{t}{K_1 + K_2.t} \quad (3)$$

where C_t represents the yield of protein in the solution (mg·g^{-1}) at time t(s), K_1 is Peleg's rate constant (s·g·mg^{-1}) and K_2 is Peleg's capacity constant (g·mg^{-1})

The rate of separation can be expressed as the time derivative of the concentration:

$$\frac{dc(t)}{dt} = R = \frac{K_2}{(K_2 + K_2 t)^2} \quad (4)$$

Therefore, the separation rate at the very beginning (mg·g^{-1}·s^{-1}) of the separation process (t=0) reduces to the reciprocal of Peleg's rate constant:

$$\frac{dc(0)}{dt} = R_0 = \frac{1}{K_1} \quad (5)$$

The maximum protein separation yield, C_e (mg·g-1) during the separation process at equilibrium time ($t \to \infty$) can be expressed as the reciprocal of Peleg's capacity constant:

$$C_e = 1/K_2 \quad (6)$$

The software, QtiPlot 5.6.1 was used to determine the values of K_1 and K_2 by non-linear regression.

Results and Discussion

Experimental protein separation kinetics data obtained at various separation conditions, was fit to a two-site model and Peleg's model. The fitting parameters for the models are listed in Tables 1 and 2 respectively.

Concentration of alkaline

Figure 1 shows the experimental protein yield (mg·g^{-1}) over time at various initial concentrations of sodium hydroxide (0.02M, 0.04M, and 0.08M) as well as the modelled protein yield C_t (mg·g^{-1}) for the two-site model and Peleg's model. Particle size (35-60 mesh) and temperature (25ºC) were kept constant.

As seen in Figure 1, the protein yield increased rapidly within 20s of mixing with sodium hydroxide followed by a gradual increase until a constant final yield (equilibrium yield) was achieved, signifying the completion of separation. The equilibrium protein yield increased, and the separation was completed more quickly, as the sodium hydroxide concentration increased. In terms of the two-site model, the protein yield constitutes protein released from a fast process (C_1) and from a slow process (C_2). As seen in Table 1, both C_1 and C_2 increased slightly between sodium hydroxide concentrations of 0.02M and 0.08M from 538.07mg·g^{-1} to 558.19mg·g^{-1} and from 82.70mg·g^{-1} to 107.99mg·g^{-1} respectively. At 0.04M sodium hydroxide concentration, the yield from the fast process decreased, however more protein was released from the slow process and the total equilibrium yield, C_{eq} showed an overall increasing trend with increasing sodium hydroxide concentration (620.77mg·g^{-1} at 0.02M to 666.19mg·g^{-1} at 0.08M). This indicates that at higher concentration, more protein was released even from intact meal particles and that the limiting effect of the slow process was reduced as alkaline concentration was increased. Since the solubility of canola protein increases with increasing alkalinity [1,3,10,35], more protein is released at higher concentrations of sodium hydroxide from all particles, thus increasing the yield from the slower process and the overall protein yield.

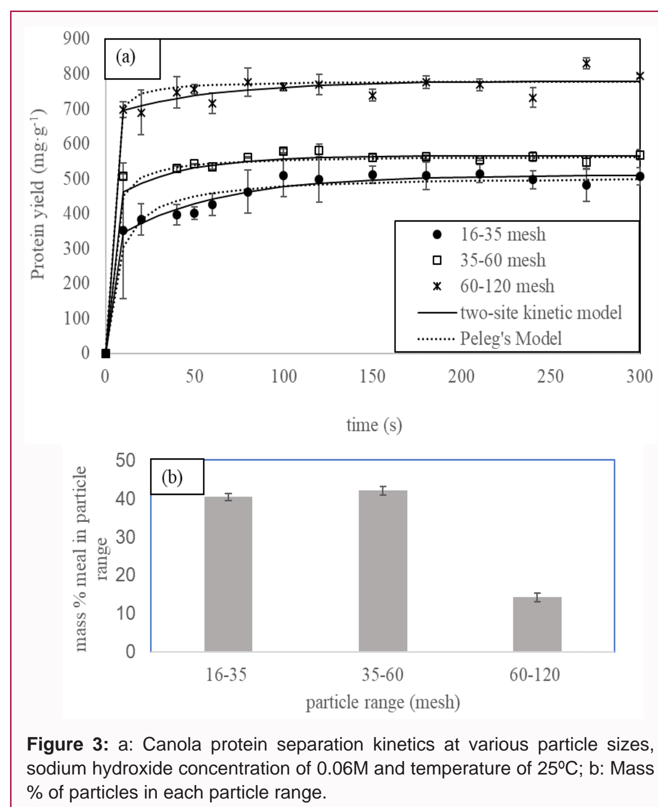

Figure 3: a: Canola protein separation kinetics at various particle sizes, sodium hydroxide concentration of 0.06M and temperature of 25ºC; b: Mass % of particles in each particle range.

The rate of separation gives an indication of how quickly the protein separation proceeds. Peleg's model enables the calculation of the rate at the very beginning of the extraction (R_o). As seen in Table 2, Ro increased dramatically from 114.67mg·g^{-1}·s^{-1} to 958.37mg·g^{-1}·s^{-1} as the sodium hydroxide concentration was increased from 0.02M to 0.08M. The maximum protein yield (C_e) also increased slightly from 626.91mg·g^{-1} to 664.64mg·g^{-1} with increasing sodium hydroxide concentration. The results indicate that a higher concentration of alkaline is favorable for protein separation from canola meal as both the separation rate and equilibrium yield increased at stronger alkaline conditions. This is consistent with the results of protein separation from plant sources [23,27,36].

Since the alkalinity of the solution plays a role in the release of protein from the meal, variation in the pH of the solution as the separation proceeds may affect the yield. The pH of the separation solution at various starting sodium hydroxide concentrations is

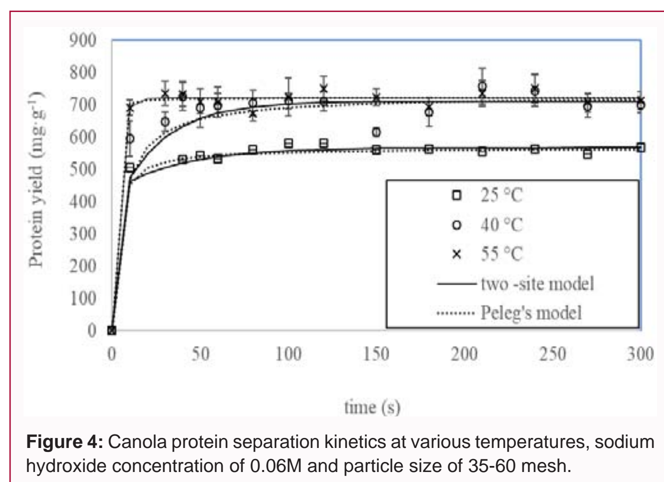

Figure 4: Canola protein separation kinetics at various temperatures, sodium hydroxide concentration of 0.06M and particle size of 35-60 mesh.

Table 1: Kinetic parameters of a two-site kinetic model for canola protein separation at various conditions.

$c_t=c_1(1-e^{-k_1 t}) + c_2(1-e^{-k_2 t});\ c_1+c_2=c_{eg}$				
Experimental condition	**C_1 (mg·g⁻¹)**	**C_2 (mg·g⁻¹)**	**C_{eq} (mg·g⁻¹)**	**R^2**
Sodium hydroxide concentration(M)[a]				
0.02	538.07	82.7	620.77	0.99
0.04	417.07	222.01	639.07	0.98
0.08	558.19	107.99	666.19	0.99
Particle size (mesh)[b]				
16-35	314.61	195.61	510.22	0.98
35-60	431.07	135.47	566.55	0.97
60-120	679.06	100.84	779.9	0.98
Temperature (°C)[c]				
25	431.07	135.47	566.55	0.97
40	367.93	342.49	710.42	0.81
55	691.5	29.17	720.67	0.99

[a]Particle size: 35-60 mesh; temperature: 25°C.
[b]Sodium hydroxide concentration: 0.06M; temperature: 25°C.
[c]Sodium hydroxide concentration: 0.06M; particle size: 35-60 mesh.

shown in Figure 2.

As seen in Figure 2, there is a drop in pH as the separation proceeds, up to the time where the equilibrium yield of protein is reached (Figure 1), after which the pH stabilizes. This indicates that there is a drop in pH with the dissolution of protein into the solution. The drop in pH is larger at a lower sodium hydroxide concentration of 0.02M. Protein dissolution occurs in solutions of high pH or extreme low pH. In alkaline solutions, the protein exhibits a net negative charge due to the loss of protons [20,25]. Therefore, the drop in pH can be attributed to the release of protons into the extraction mixture. The drop in pH at higher concentrations of alkaline (0.04M and 0.08M) is not large. This implies that there is an excess hydroxide concentration, which is not affected greatly by the release of protons into the extraction mixture, whereas in the case of 0.02M sodium hydroxide concentration, there is a depletion of hydroxide ions, causing a drop in pH. Therefore, stronger alkaline conditions are favorable for maintaining the alkalinity of the solution in excess, to ensure that the rate and yield of the separation are not significantly affected by pH drop.

Particle size

After defatting, the canola meal was sieved into three particle

Table 2: Kinetic parameters of Peleg's model for canola protein separation at various conditions.

$C_t = \dfrac{t}{K_1 + K_2 t};\ R_0 = \left(\dfrac{1}{K_1}\right);\ C_e = \left(\dfrac{1}{K_2}\right)$			
Experimental condition	**R_o(mg·g⁻¹·s⁻¹)**	**C_e(mg·g⁻¹)**	**R^2**
Sodium hydroxide concentration (M)[a]			
0.02	114.67	626.91	0.96
0.04	281.39	649.29	0.98
0.08	958.37	664.64	0.98
Particle size (mesh)[b]			
16-35	77.15	509.44	0.96
35-60	223.69	566.56	0.96
60-120	769.98	780.76	0.94
Temperature (°C)[c]			
25	223.69	566.56	0.96
40	128.52	730	0.79
55	2858.42	722.35	0.99

[a]Particle size: 35-60 mesh; temperature: 25°C.
[b]Sodium hydroxide concentration: 0.06M; temperature: 25°C.
[c]Sodium hydroxide concentration: 0.06M; particle size: 35-60 mesh.

sizes namely 16-35 mesh (1.19mm–0.5mm), 35-60 mesh (0.5mm–0.25mm) and 60-120 mesh (0.25mm–0.125mm). Figure 3 shows the modelled and experimental protein yield for various particle ranges (Figure 3a) and the mass % of particles in each range (Figure 3b). Sodium hydroxide concentration (0.06M) and temperature (25°C) were kept constant.

As seen in Figure 3a, the protein yield increased as the particle size decreased, with 60-120 mesh particles giving the largest final yield. Based on the two-site model, the protein yield from the fast process, C_1 increased, whereas the protein yield from the slow process, C_2 decreased with decreasing particle size (Table 1). The equilibrium protein yield, C_{eq} also increased from 510.22mg·g⁻¹ for 16-35 mesh particles, to 779.90mg·g⁻¹ for 60-120 mesh particles, which was the largest yield among all separations conducted. This accounts for a 269.68mg·g⁻¹ increase in yield. This can be attributed to the increase in the number of broken particles with decreasing particle size, which enable easy access of the solvent to the protein on the particle surface and therefore contribute to the faster release of protein [37]. Therefore, a majority of the protein in the smaller particle ranges is released from the faster process. Peleg's model shows an increase in both the initial rate of protein release R_o, (from 77.15mg·g⁻¹·s⁻¹ to 769.98mg·g⁻¹·s⁻¹) and the maximum protein yield, C_e (509.44mg·g⁻¹ to 780.76mg·g⁻¹) with decreasing particle size. This indicates that a smaller particle size is favorable for protein separation as more protein is released at a faster rate. The particles in the smallest particle range of 60-120 mesh constitute 14% of the total meal (Figure 3b). Larger particles (16-35 mesh and 35-60 mesh) constitute a majority of the meal (40% and 42% respectively), therefore in a commercial context, grinding of the meal to a smaller particle size may be more favorable for larger overall yields from the total meal. The results indicate that the smallest particle range of 60-120 mesh, is most favorable for protein separation in terms of rate and yield, as the presence of smaller broken cells contributes to the faster release of protein. Bigger particle ranges may contain fewer broken down cells and more intact particles, thus limiting protein mass transfer and consequently the rate and yield. Smaller particles also provide a greater surface area for

contact with the solvent [24,37]. Since separation rate is influenced by the available surface area for reaction, more protein is separated from smaller particles, whereas less surface area is available in bigger particles causing a slower release of protein.

Temperature

Protein was separated at three different temperatures (25°C, 40°C and 55°C). Figure 4 shows the modelled and experimental separated protein yield for various temperatures. Particle size (35-60 mesh) and sodium hydroxide concentration (0.06M) were kept constant.

The two-site model indicates that at room temperature (25°C), the faster process releases more protein (C_1= 431.07mg·g^{-1}) than the slow process (C_2=135.47mg·g^{-1}) (Table 1). At 40°C, both processes contribute similar amounts of protein (C_1=367.93mg·g^{-1} and C_2=342.49mg·g^{-1}) and at 55°C, only 29.17mg·g^{-1} is released by the slow process. This is only 4% of the total protein released, indicating that a majority of the protein is released at a faster rate and the rate limiting effect of the slow process is reduced at 55°C. This is confirmed by the initial rate, (R_o) value in Peleg's model (Table 2). While the maximum protein yields (C_e) at 40°C and at 55°C are very similar, the rate of protein release is much higher at 55°C (Tables 1 and 2). There was a drop in the initial rate at the intermediate temperature of 40°C as the slower process contributed to more protein release. The initial rate increased from 223.69mg·g^{-1}·s^{-1} at 25°C to more than 10 times the value (2858.42mg·g^{-1}·s^{-1}) at 55°C. The results indicate that a higher temperature of 55°C is favorable for greater yields of protein from canola meal. Similar results were obtained by other researchers, who pointed out that an increase in temperature increases the internal mass transfer within the solid, thereby releasing protein at a faster rate [24,38].

Conclusion

Protein yield and reaction rate increased with increasing alkaline concentration, increasing temperature and decreasing particle size. Change in particle size had the greatest effect on yield. A particle size of 60-120 mesh gave the largest equilibrium yield of 779.90mg·g^{-1} and a 269.68mg·g^{-1} increase in yield was observed between particle sizes 16-35 mesh and 60-120 mesh. At a higher temperature of 55°C, the faster process dominated the rate and protein was released at an initial rate of 2858.42mg·g^{-1}·s^{-1}, which was a tenfold increase from room temperature.

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