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Kinetics of Egg-Yolk Protein Hydrolysis and Properties of Hydrolysates

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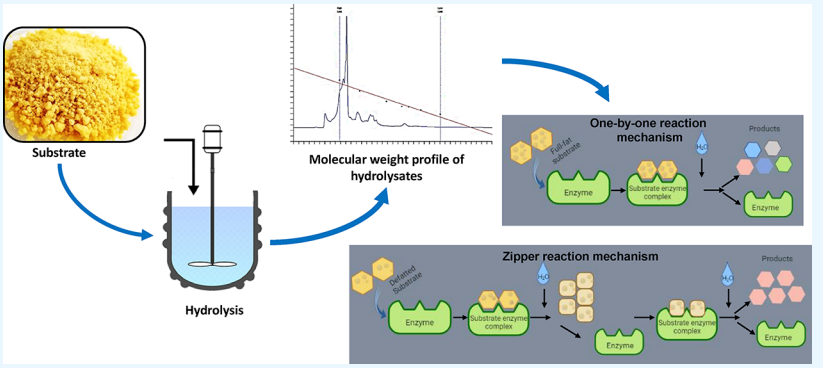

ABSTRACT: Lecithin-free egg yolk (LFEY) is a byproduct of the extraction of egg-yolk phospholipids, which contain approximately 46% egg yolk proteins (EYPs) and 48% lipids. The enzymatic proteolysis is the alternative to increase the commercial value of LFEY. The kinetics of proteolysis in full-fat and defatted LFEY with Alcalase 2.4 L was analyzed in terms of the Weibull and Michaelis−Menten models. A product inhibition effect was also studied in the full-fat and defatted substrate hydrolysis. The molecular weight profile of hydrolysates was analyzed by gel filtration chromatography. Results pointed out that the defatting process did not importantly affect the maximum degree of hydrolysis (DH_{max}) in the reaction but rather the time at which DH_{max} is attained. The maximum rate of hydrolysis (V_{max}) and the Michaelis−Menten constant K_M were higher in the hydrolysis of the defatted LFEY. The defatting process might have induced conformational changes in the EYP molecules, and this affected their interaction with the enzyme. Consequently, the enzymatic reaction mechanism of hydrolysis and the molecular weight profile of peptides were influenced by defatting. A product inhibition effect was observed when adding 1% hydrolysates containing peptides lower than 3 kDa at the beginning of the reaction with both substrates.

1. INTRODUCTION

Enzymatic hydrolysis is a widely used alternative to add value to protein wastes and byproducts. The properties of hydrolysates obtained from this process depend on several factors such as the substrate, type of enzyme, and process conditions. The reaction conditions as well as the nature of the substrate and the enzyme directly influence the characteristics of the hydrolysates. Foaming and emulsifying properties as well as increased solubility are the most reported in enzymatic protein hydrolysis reactions. The hydrolysates' molecular size, hydrophobicity, and polar groups can also vary depending on the hydrolysis conditions.[1] The preferred substrates studied so far have been proteins from animal or vegetable wastes or byproducts from different industries.[2−12]

Most of the studies from the literature are focused on obtaining hydrolysates with improved functional properties or bioactive peptides that can be used in biomedical or food applications.[13−18] However, only a few studies have been found on the kinetics of protein hydrolysis reactions and these are focused mainly to identify enzyme inhibition, which is a phenomenon frequently observed during protein hydrolysis reactions. Valencia et al.[19] determined product inhibition by hydrolyzing fish muscle protein with Alcalase. Demirham et al.[20] reported an uncompetitive inhibition in the hydrolysis of sesame protein by Alcalase. Sousa et al.[21] hydrolyzed whey protein with immobilized Alcalase and observed competitive inhibition byproducts. Kammoun et al.[22] used Neutrase for the hydrolysis of wheat proteins and reported a competitive−uncompetitive inhibition type. No reports have been found on the kinetics of reactions conducted under high substrate concentrations and

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different substrate fat contents. Hence, most researchers[4,23,24] work with defatted proteins, but the possible role of fat or the defatting process on the reaction kinetics and the final properties of hydrolysates has not been studied. This might be because the fat content of raw materials such as vegetable proteins, fish, and peanuts is low; however, there are byproducts, such as with the lecithin-free egg yolk that can have up to 48% fat. Such a high-fat concentration in the raw material could interfere with the mechanism followed by the enzyme during the hydrolysis reaction and even modify the final properties of hydrolysates. Therefore, in this work, the role of fat and/or the defatting process on the kinetics of a lecithin-free egg-yolk protein−Alcalase system was studied. The properties of hydrolysates (concentration and molecular weight of peptides) were related not only to the substrate used (full-fat or defatted LFEY) during the reaction but also to the degree of hydrolysis. The product inhibition effect in the hydrolysis reaction with both substrates was also studied.

2. THEORY

The enzymatic hydrolysis of natural protein sources is a complex reaction at the molecular level due to the specificity of the enzymes and the composition of the substrates involved.[25] Several parametric models have been used in literature to study the kinetics of the enzymatic reactions of different enzyme−substrate systems.

The hydrolysis of whey protein with trypsin was analyzed using the Elovich equation to estimate the initial reaction rates in batch experiments with up to a 22% w/w protein concentration.[26]

$$\frac{dX}{dt} = a_1 \frac{E_0}{S_0^2} e^{[-a_2 X]} \tag{1}$$

The Elovich (eq 1) is a robust model with only two parameters and is well suited to describe reaction systems with strong product inhibition and low catalyst deactivation rates.

Marquez et al.[27] studied the proteolysis of hemoglobin−Alcalase 0.6 L and used eq 2 to describe the kinetics of this reaction under different conditions

$$\frac{d(DH)}{dt} = a e^{[-b(DH)]} \tag{2}$$

where a and b group the following parameters

$$a = \frac{k_2 e_0}{s_0} \text{ and } b = \frac{k_3 K_M}{k_2}$$

From these, k_2 is the reaction rate constant, k_3 is the reaction rate constant for inactivation, K_M is the Michaelis−Menten constant, e_0 is the initial enzyme concentration, and s_0 is the initial substrate concentration. Equation 2 describes the hydrolysis as a zero-order reaction with simultaneous second-order inactivation of the enzyme.

Sousa et al.[21] studied the enzymatic hydrolysis of whey proteins using immobilized Alcalase on an agarose gel. They fitted the experimental data to a Michaelis−Menten kinetic model with competitive inhibition of the product (eq 3)

$$V = -\frac{kEN}{K_M\left(1 + \frac{I}{K_I}\right) + N} \tag{3}$$

where I is the molar concentration of the inhibitor, N is the molar concentration of peptide bonds in the substrate that can be hydrolyzed by Alcalase, and k, K_M, and K_I are the kinetic parameters of the model.

Ruan et al.[28] investigated the characteristic reaction kinetics of enzymatic hydrolysis of egg-white protein with pepsin. They used an empirical kinetic model (eq 4) to predict the course of protein hydrolysis at different reaction times.

$$DH = \frac{1}{b}\ln(1 + abt) \tag{4}$$

This model was also used to describe the kinetics of hydrolysis of sorghum protein with Alcalase.[29] The parameters a and b were determined from graphs of the degree of hydrolysis as a function of time. The parameter a was then plotted as a function of the initial enzyme-to-substrate concentration ratio, e_0/S_0, to obtain a straight line from which the rate constant (k_2) and $k_2 S_0$ were determined as the slope and the intercept, respectively.

Vázquez et al.[30] optimized the experimental conditions to produce fish protein hydrolysates using Alcalase and used the Weibull equation (eq 5) to analyze the reaction kinetics

$$H = H_m\left\{1 - e\left[-\ln 2\left[\frac{t}{\tau}\right]^{\beta}\right]\right\} \text{ with } \nu_m = \frac{\beta H_m \ln 2}{2\tau} \tag{5}$$

where H is the hydrolysis degree (%), t is the hydrolysis time (min), H_m is the maximum hydrolysis degree (%), β is a dimensionless parameter associated with the slope of the hydrolysis process, τ is the time needed to reach the semi-maximum degree of hydrolysis (min), and ν_m is the maximum rate of hydrolysis.

3. MATERIALS AND METHODS

3.1. Lecithin-Free Egg Yolk. The raw material, namely, lecithin-free egg yolk (LFEY), was supplied by Fresenius Kabi Deutschland GmbH. This byproduct comes from the lecithin extraction process. It contains approximately 48% lipids (triglycerides, free fatty acids, and cholesterol) and approximately 45% proteins. The protein composition of avian egg yolk is complex. Using mild centrifugation, egg yolk can be separated into two main fractions: plasma and granules. The plasma is approximately 77−81% yolk dry matter, whereas granules are 19−23% yolk dry matter. The proteins from plasma are 85% low-density lipoproteins (LDL) and 15% livetins. On the granule side, the proteins are 70% high-density lipoproteins, 16% phosvitin, and 12% LDL.[31] The protein composition is dependent on factors such as the feed intake and environment during the hen's productive life. The egg lipids and protein content vary throughout the productive cycle of the hen.[32]

3.2. Materials. The chemicals and reagents used for analysis included di-sodium hydrogen phosphate dihydrate from Sigma-Aldrich, Kjeldahl catalyst tablets having 3.5 g of K_2SO_4 and 0.4 g of $CuSO_4$ per tablet (FOSS), sodium hydroxide, boric acid, and sulfuric acid (Meyer, México). The enzyme used was Alcalase 2.4 L purchased from Sigma and it was kept stored at 4 °C, and it is an endo-protease enzyme of *Bacillus subtilis*. This enzyme is a serine protease.

3.3. Hydrolysis Reaction. The substrate was hydrated in phosphate buffer, the pH was set to 8.0 with NaOH (1 N) solutions. Experiments were performed in an 80 mL stirred reactor fitted with pH and temperature controls. The reaction was initiated when the enzyme was added. The protein hydrolysis was followed by the pH-stat technique.[33] NaOH (2 N) solution was used to control the medium pH. Hydrolysate samples were freeze-dried for further analysis. All experiments

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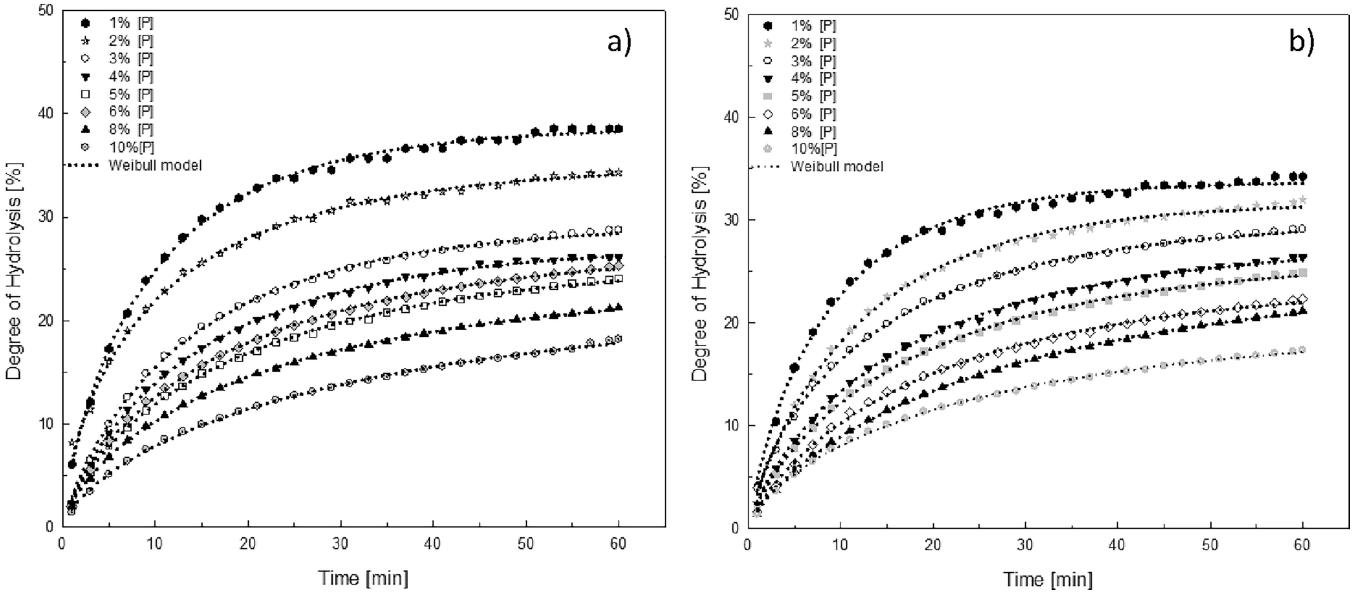

Figure 1. Kinetics of the degree of hydrolysis of lecithin-free egg yolk (LFEY) proteins with Alcalase as a function of the protein concentration and fitting of the Weibull model to experimental data: (a) defatted LFEY and (b) full-fat LFEY. Reaction conditions: pH 8, 55 °C, and 0.5% (*v*/*v*) Alcalase.

were conducted at least by duplicate and the reproducibility between experiments was within ±5%.

3.4. Degree of Hydrolysis. The degree of hydrolysis (DH) or the ratio between the number of cleaved and total peptide bonds was estimated according to the pH-stat method where the DH is linearly dependent on the volume of the base added to keep the pH constant during hydrolysis, according to eq 6[31]

$$\mathrm{DH}\ (\%) = \frac{BN_b}{M_p \alpha h_{\mathrm{Tot}}} \tag{6}$$

where B is the base consumption (mL), N_b is the base normality, α is the average degree of dissociation of the α-NH_2 groups in the protein substrate, M_p is the protein mass (g) ($N \times 6.62$), and h_{Tot} is the total number of peptide bonds used for proteolytic hydrolysis (7.33 meq/g).

3.5. Reaction Kinetics Experiments. The hydrolysis of the lecithin-free egg yolk protein was conducted under different initial substrate concentrations (1, 2, 3, 4, 5, 6, 8, and 10 g/100 mL) with both substrates (defatted and full-fat egg yolk proteins). The following reaction conditions were kept constant: pH 8, 55 °C, and enzyme concentration [E] of 0.5% (*v*/*v*) during the 60 min of reaction. Samples were taken at 10, 20, 40, and 60 min lyophilized and further analyzed by gel filtration chromatography to determine the molecular size distribution of peptides in the range of 12,327−238 Da.

Enzymatic hydrolysis reactions were also carried out at a constant initial protein concentration (10% *w*/*v*) and stopped at different reaction times, namely, 20, 40, 100, and 180 min, with both full-fat and defatted EY proteins. The hydrolysates harvested from these reactions were lyophilized and used to evaluate the product inhibition effect in further reactions. The hydrolyzed protein (0.5 or 1% *w*/*w*) was added to supplement the total initial protein concentration (10% *w*/*v*) in hydrolysis reactions with full fat and defatted substrates. The pH was kept constant at 8.0 with 2 N sodium hydroxide solution in all reactions. The initial reaction rate (V_0) was estimated according to[23] (eq 7) with some modifications

$$V_0 = \frac{\mathrm{DH}_{10\mathrm{min}} \times S_0 \times 60}{5 \times 100} \tag{7}$$

where $\mathrm{DH}_{10\mathrm{min}}$ was obtained from the slope in the first 10 min of reaction and S_0 is the initial protein concentration (g/L).

For the determination of the kinetic parameters, the maximum rate of hydrolysis (V_{max}), and the constant (K_m), the non-linear fit of the Michaelis−Menten model (eq 8) to the experimental data was performed using SigmaPlot version 14.5 (Systat Software Inc.).

$$V_0 = \frac{V_{\mathrm{max}}[S_0]}{K_m + [S_0]} \tag{8}$$

In addition, the Weibull model (eq 9) was fitted to experimental results to analyze the hydrolysis reaction kinetics also using a non-linear approach in SigmaPlot.

$$\mathrm{DH} = \mathrm{DH}_m\left[1 - \exp\left(-\frac{t}{\mathrm{MDHT}}\right)^{\beta}\right] \tag{9}$$

where DH_m is the maximum degree of hydrolysis, MDHT, is the medium degree of hydrolysis time (min), β is a dimensionless parameter associated with the slope of the hydrolysis, and t is the hydrolysis time.

3.6. Inhibition Ratio. The inhibition ratio (IR_t) of reaction products was evaluated in terms of the hydrolysis degree at a specific reaction time (t), employing the following equation (eq 10)[22]

$$\mathrm{IR}_t(\%) = \frac{\mathrm{DH}_t - \mathrm{DH}_{It}}{\mathrm{DH}_t} \tag{10}$$

where DH_t and DH_{It} is the degree of hydrolysis measured at the time t (min) without and with the inhibitor, respectively.

3.7. Molecular Weight Profile of Hydrolysates. The peptide profile of hydrolysates was determined using size exclusion chromatography (SEC-HPLC) with a PL-Aquagel-OH 20 column. The detection was carried out at a 220 nm wavelength. The column was calibrated with cytochrome C (12,327 Da), insulin chain B (3495. 89 Da), and an HPLC

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peptide standard mixture from Sigma-Aldrich (1046, 573, 555, 379, and 238 Da) using water as the mobile phase.

4. RESULTS AND DISCUSSION

4.1. Hydrolysis Reaction Kinetics. The kinetics of the hydrolysis reaction was studied by using defatted LFEY (4% fat *v*/*v*) and full fat LFEY (48% fat *v*/*v*) byproducts as substrates. The response variable of the reaction was the degree of hydrolysis. Results showed that increments in the protein concentration from 1 to 10% *w*/*v* decreased by more than 50% of the degree of hydrolysis after 60 min of reaction with both substrates of full-fat and defatted LFEY proteins. However, the fall in the DH was slightly higher (53%) when the reaction was conducted with the defatted substrate regarding that (50%) observed with the full-fat LFEY byproduct (Figure 1). The Weibull equation fairly describes the DH profiles as shown in Figure 1. The maximum degree of hydrolysis (DH_{max}), and the mean degree of hydrolysis time (MDHT) obtained from the Weibull model are summarized in Table 1.

Table 1. Weibull Equation Parameters Were Obtained by Fitting the Degree of Hydrolysis Profiles from Reactions Conducted under Different Initial Protein Concentrations with Both Full-Fat and Defatted Lecithin-Free Egg Yolk (LFEY)

	defatted LFEY			full-fat LFEY		
protein (% *w*/*v*)	DH_{max}	MDHT (min)	β	DH_{max}	MDHT (min)	β
1	38.79	9.71	0.80	33.79	8.83	0.86
2	35.40	10.53	0.69	31.83	12.11	0.88
3	29.33	14.06	0.86	30.27	13.78	0.77
4	27.39	15.08	0.84	27.81	16.97	0.82
5	26.24	19.38	0.77	26.47	18.09	0.82
6	27.20	18.37	0.79	24.17	20.55	0.83
8	24.09	22.32	0.74	24.99	28.03	0.80
10	22.44	32.15	0.73	20.02	24.67	0.74

Data from Table 1 shows that DH_{max} decreased and the MDHT was longer as the initial protein concentration increased in the reaction with both full-fat and defatted LFEY. The decrease in the DH_{max} was approximately 42% in the whole range of protein concentrations tested with both substrates, but the MDHT value was much longer in the hydrolysis of the deffated LFEY. Such a result suggests that some conformational changes were induced to the egg-yolk protein molecules during the defatting process, affecting the interaction between the defatted egg-yolk proteins and the enzyme Alcalase.

The initial reaction rate (V_0) was evaluated according to eq 7. Results show that V_0 rise with increments in the initial protein concentration. Hence, the protein molecules transformed per minute in the reaction were higher the higher the initial protein concentration (Figure 2). These results follow the Michaelis–Menten model as confirmed by the fair fitting of eq 8 to the experimental data from both full-fat and defatted LFEY proteins (Figure 2). The kinetic parameters V_{max} and K_m determined by non-linear regression of the Michaelis–Menten model to the data are summarized in Table 2 and compared to those reported in the literature for the hydrolysis of different proteins with the same enzyme (alcalase). The V_{max} and K_m values obtained for the hydrolysis of defatted LFEY were higher than those observed for the hydrolysis of full-fat substrate. This result suggests that the kinetics of the hydrolysis of egg yolk proteins with Alcalase

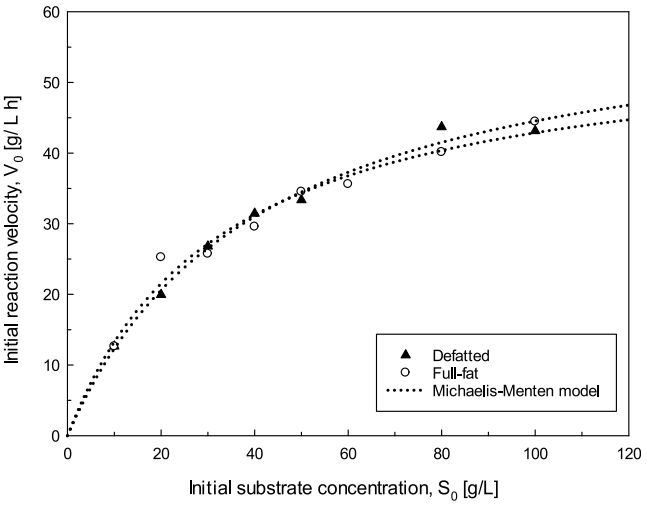

Figure 2. Kinetics of the hydrolysis of full-fat and defatted egg-yolk proteins according to the Michaelis–Menten model (eq 8). Reaction conditions: pH 8, T = 55 °C, and [E] = 0.5% (*v*/*v*).

Table 2. Kinetic Parameters of Michaelis–Menten Model for the Hydrolysis of LFEY and Other Proteins with Alcalase[a]

reference	protein	V_{max} (g/L h)	K_m (g/L)
this work	defatted LFEY	62.8	40.99
	full-fat LFEY	57.02	32.97
23	globulins	18.7	10.7
	albumins	21.3	20
20	sesame protein	126.6	57.63

[a]LFEY: lecithin-free egg yolk.

was modified by the fat extraction process used to reduce fat from this byproduct.

The kinetic parameters obtained in this work are in the range of those reported in the literature with Alcalase even though the reaction conditions used by other researchers were quite different from those from this work (Table 2). This confirms that the Alcalase enzyme is a versatile biocatalyst that keeps its proteolytic capacity for different substrates and reaction conditions.[34]

According to the Michaelis–Menten model, the higher the K_m, the lower the affinity of the enzyme for the substrate.[35] The K_m value was smaller in the hydrolysis of full-fat egg yolk proteins (EYP) compared to the K_m obtained from the hydrolysis of defatted EYP. The Alcalase–full-fat EYP intermediary complex seems to be more strongly bound than the Alcalase–defatted EYP complex. This might be due to changes in the molecular conformation of the defatted egg-yolk protein molecules induced by the defatting process, which involves the use of an organic solvent (hexane). Hexane is a nonpolar solvent that might have exposed amino acid residues in the defatted LFEY that were not visible before, and this might affect the hydrolysis reaction mechanism followed by the enzyme concerning that followed in the reaction with the full-fat LFEY substrate.

To get further insight into these effects, hydrolysates obtained under the different protein concentrations in full-fat and defatted egg-yolk proteins were analyzed using gel filtration chromatography (GFC).

4.2. Molecular Size (MW) Distribution of Hydrolysates. The GFC analysis of hydrolysate samples taken after 10 min of

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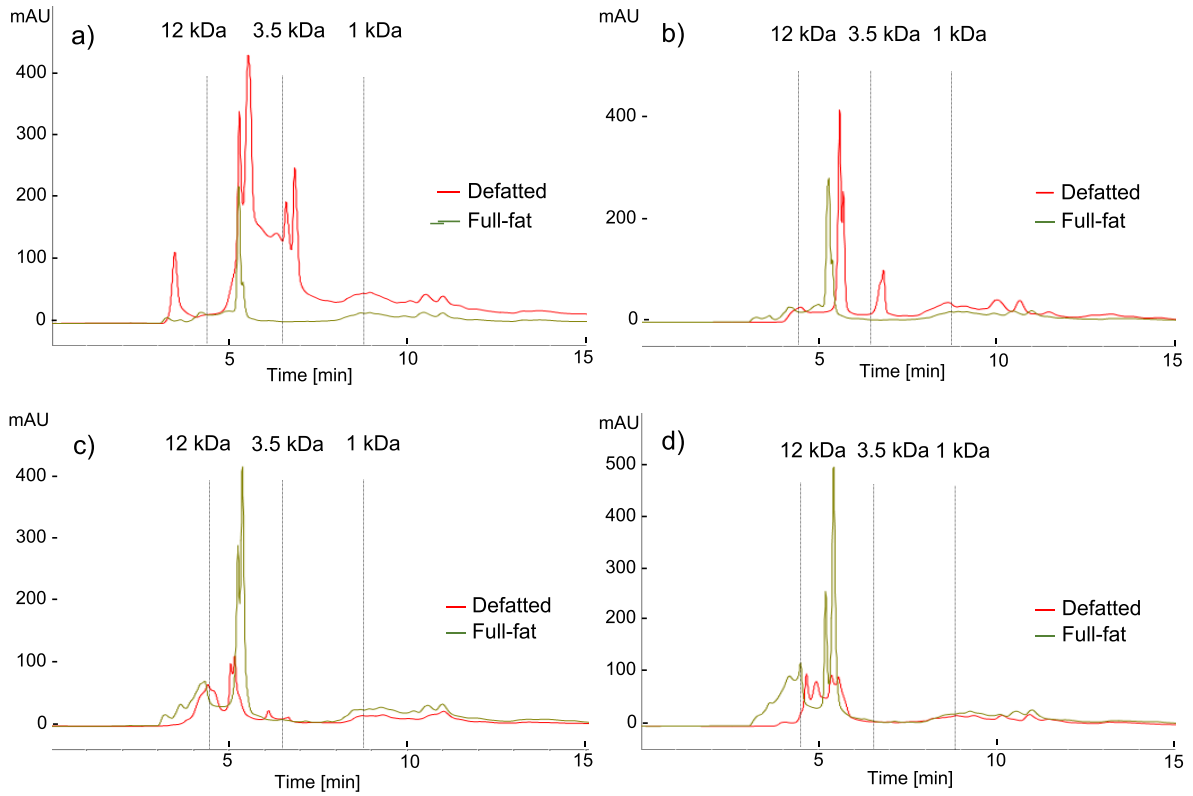

Figure 3. Molecular size distribution by gel filtration chromatography of hydrolysates obtained at 10 min of reaction with different fat and protein concentrations: (a) 1% protein, (b) 2% protein, (c) 6% protein, and (d) 10% protein.

reaction suggest that the concentration of both full-fat and defatted egg-yolk proteins in the reaction mixture influenced the molecular weight (MW) and concentration of peptides obtained in the hydrolysates (Figure 3).

Figure 3a shows that, in the hydrolysis of 1% defatted protein, the number and intensity of peaks are the highest in the whole range of the molecular weight analyzed. This suggests that, in the first 10 min of the reaction, the concentration of the hydrolysis products is also the highest in the analyzed molecular-weight range. However, the intensity and the width of peaks decreased as the concentration of the defatted EYP increased, and this indicates that the hydrolysis reaction to produce peptides in this MW range takes longer, that is, more than 10 min. Otherwise, hydrolysates from the full-fat EYP showed that the intensity of the peaks from 10 min samples increased in the range 3.5 kDa > MW < 12 kDa with increments in the protein concentrations. These results point out that, even though the degree of hydrolysis at this reaction time was similar in both substrates, the compositions of their hydrolysates are not. This might also indicate that the reaction mechanism followed by the enzyme in the hydrolysis of the full-fat LFEY proteins is different from that followed in the hydrolysis of the defatted substrate.

The MW profile of hydrolysates from the hydrolysis of full-fat egg-yolk proteins from Figure 4 shows that proteins are hydrolyzed into the same mixture of peptides since the beginning of the reaction, but the peptide concentration increases with time and the degree of hydrolysis. This behavior points out that the enzyme followed the one-by-one reaction mechanism in this reaction.[33] Otherwise, in the hydrolysis of defatted EY proteins (Figure 5), the protein is rapidly hydrolyzed into a large population of peptides, which are then hydrolyzed into smaller peptides, and therefore the composition of hydrolysates changes with time and the increase in the degree of hydrolysis. This behavior is better described by a zipper reaction mechanism.[33]

These results are aligned with the differences observed in the kinetic parameters of the hydrolysis reactions from the full-fat and the defatted egg-yolk proteins.

The decrease in the DH observed with increments in the concentration of egg-yolk proteins (Figure 1) might also be an indication of enzyme inhibition by the reaction products (i.e., peptides). To explore this possibility, small concentrations (0.5 and 1% *w*/*v*) of hydrolysates harvested at different reaction times were added at the beginning of hydrolysis reactions.

4.3. Product Inhibition Effects on the Hydrolysis of LFEY Proteins. The inhibition by the reaction products in proteolysis reactions has been suggested in the literature by several researchers.[19,21,22,26] In this work, this effect was studied by supplementing 0.5 and 1% *w*/*v* the total initial protein concentration (10% *w*/*v*) with lyophilized hydrolysate samples harvested at different hydrolysis times (20, 40, 100, and 180 min). These experiments were carried out with both full-fat and defatted LFEY proteins and by keeping constant other reaction conditions (pH 8, 55 °C, and 0.5% *w*/*v* enzyme concentration). It was observed that supplementing the total initial protein concentration with 0.5% *w*/*v* hydrolysate samples harvested at 20 and 40 min of hydrolysis did not affect the DH profiles in the hydrolysis of both full-fat and defatted egg-yolk proteins (Figure 6).

However, when adding 0.5% *w*/*v* lyophilized hydrolysates harvested at longer reaction times (100 and 180 min), the degree of hydrolysis decreased since the first minutes of reaction in both substrates. These results suggest an inhibition effect induced by the products found in these hydrolysate samples,

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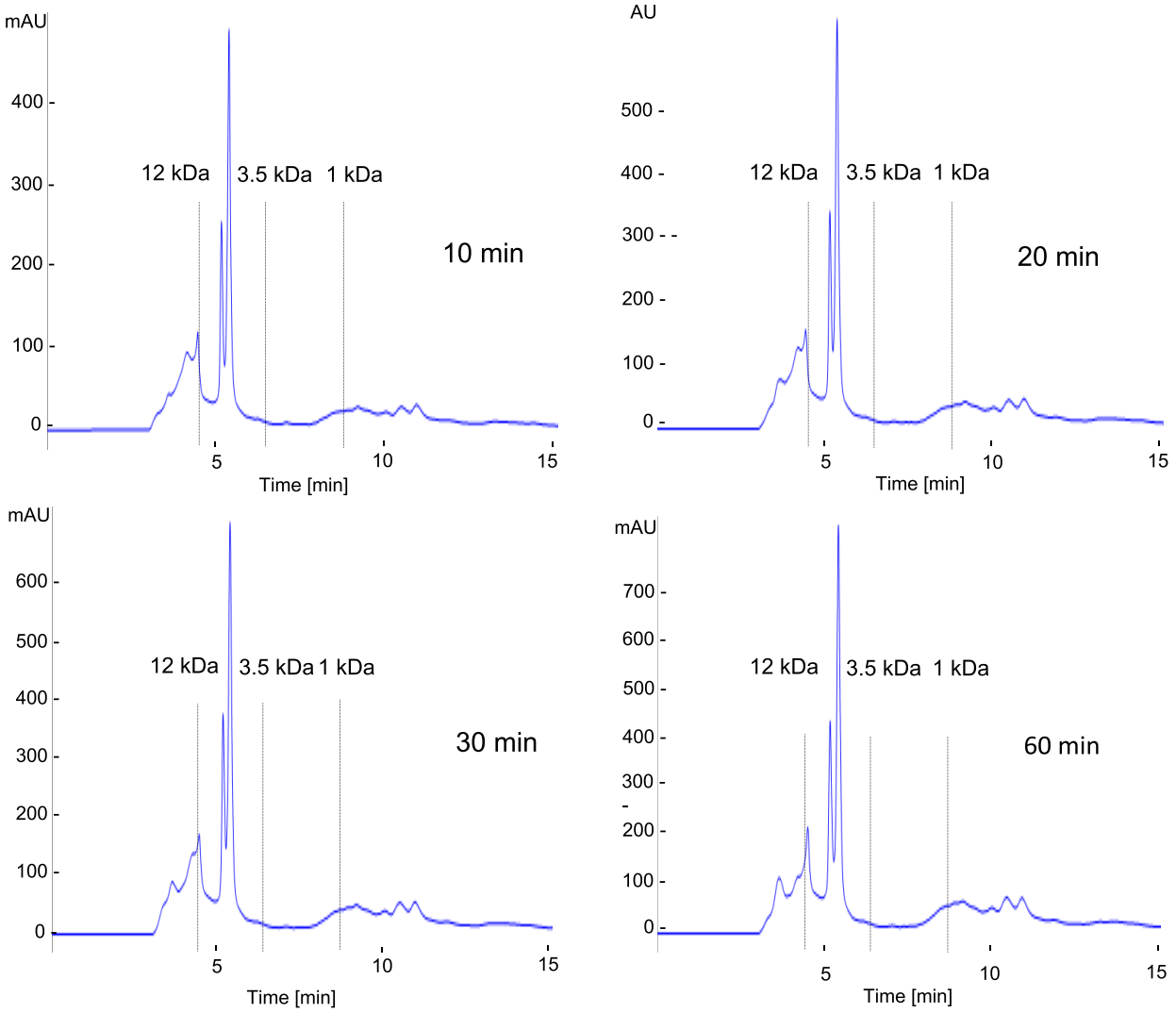

Figure 4. Molecular size profile of hydrolysate samples taken along the hydrolysis reaction of 10% full-fat egg-yolk proteins.

which probably are peptides smaller than those produced earlier in the hydrolysis reaction. This effect was similar when adding 1% of hydrolysates collected at different reaction times in the hydrolysis of full-fat egg-yolk protein (Figure 7a).

These results also point out that product inhibition was probably caused by the small peptides found in the hydrolysates after 3 h of reaction. This inhibition effect was quite similar in both substrates of defatted and full-fat egg-yolk proteins.

However, in the hydrolysis of defatted egg-yolk proteins, a slight increase in the DH occurred after 60 min of reaction when adding 1% *w*/*w* hydrolysate harvested after 20 min (Figure 7b). Additionally, an increasing fall in the DH was observed when 1% *w*/*w* hydrolysate harvested at 40 min of reaction was added to supplement the total initial protein concentration of the defatted substrate (Figure 7b). The largest decrease in the DH occurs when hydrolysates obtained after 180 min of reaction is added to supplement the initial protein concentration in the hydrolysis reactions of both full-fat and defatted EY proteins. As pointed out before, it is expected that the size of peptides found in these hydrolysates is the smallest. The inhibition ratio (eq 10) was estimated under all tested conditions and is summarized in Table 3.

Results confirm that the largest inhibition effect occurred when both 0.5% and 1% hydrolysates harvested at 180 min of reaction were added.

4.4. Effect of the Reaction Inhibition on the MW Distribution of Hydrolysates. The hydrolysates collected at 180 min of reaction showed the highest inhibition effect in the hydrolysis reaction of both full fat and defatted egg-yolk proteins (Table 3). The molecular size profiles of hydrolysates obtained from the hydrolysis of both substrates carried out with and without the addition of 1% *w*/*w* hydrolyzed protein harvested at 180 min of reaction are compared in Figure 8.

The MW profiles of hydrolysates obtained from the supplemented and non-supplemented full-fat LFEYs are quite similar (Figure 8a). The main difference (from left to right in Figure 8a) is a small shoulder indicating the presence of peptides larger than 12 kDa, a slightly higher concentration of approximately 5 kDa peptides, and the presence of products having MW ≈ 1 kDa and MW ≪ 1 kDa compared to the profile from the non-supplemented reaction.

Otherwise, in the defatted substrate, two main changes were induced by supplementing the total initial protein concentration with 1% *w*/*w* of the hydrolyzed protein. The first one is the appearance of a 12 kDa peak, and the second one is the presence of another peak around 2 kDa in the supplemented hydrolysis

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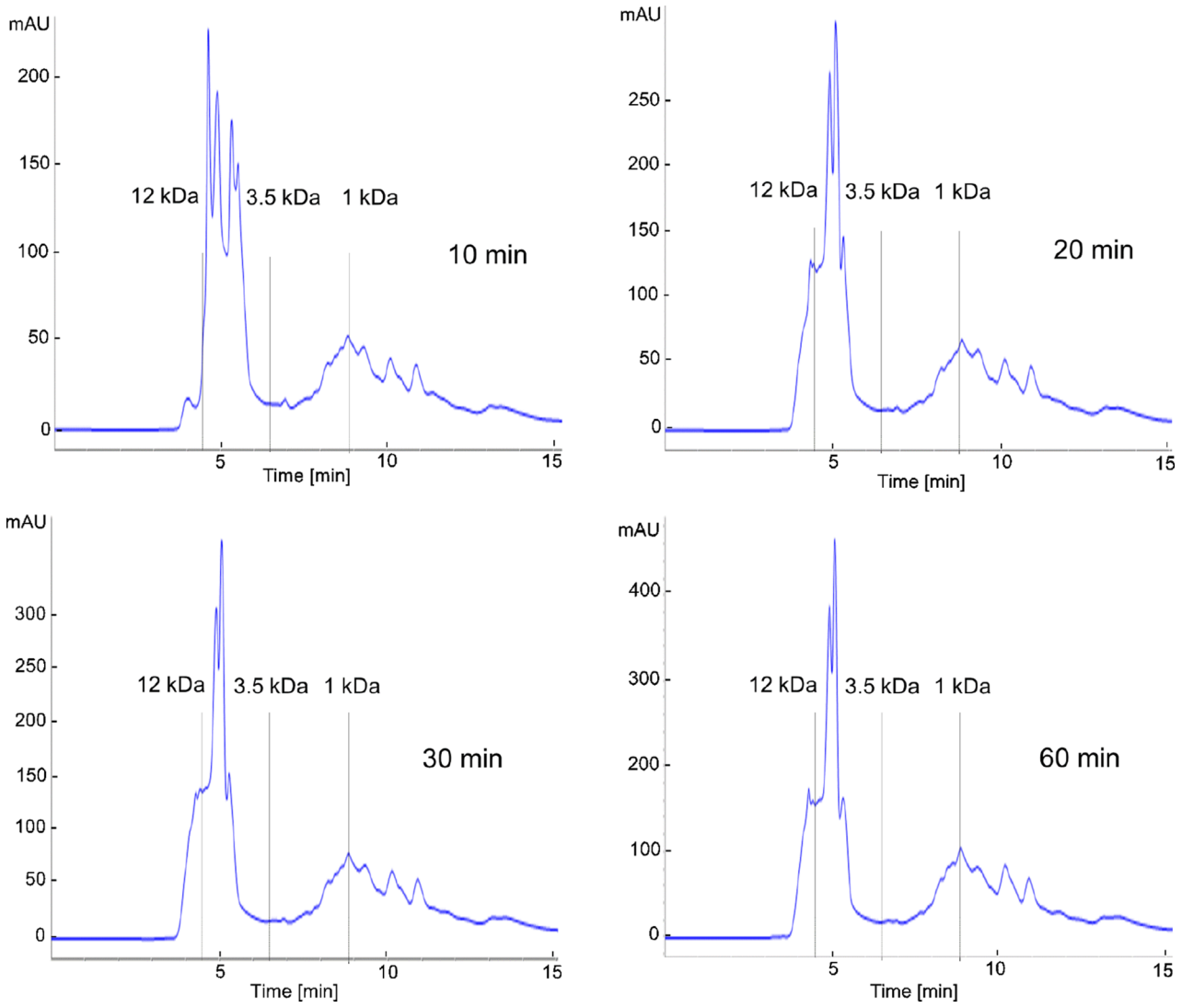

Figure 5. Molecular size profile of hydrolysate samples taken along the hydrolysis reaction of 10% defatted egg-yolk proteins.

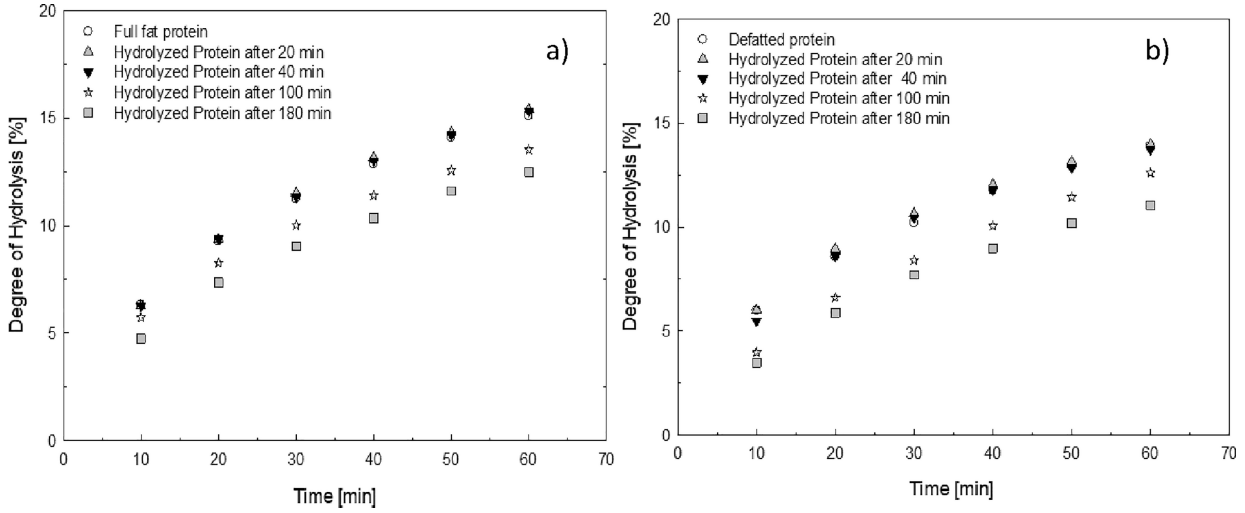

Figure 6. Effect of supplementing by 0.5% *w*/*v* the total initial protein concentration with hydrolysates harvested at different reaction times on the kinetics of hydrolysis of (a) full fat and (b) defatted LFEY proteins.

(Figure 8b). Such results indicate that small peptides detected between 1 and 2 kDa might be responsible for the fall in the degree of hydrolysis and probably the inhibition in the supplemented reactions with both substrates. The inhibition

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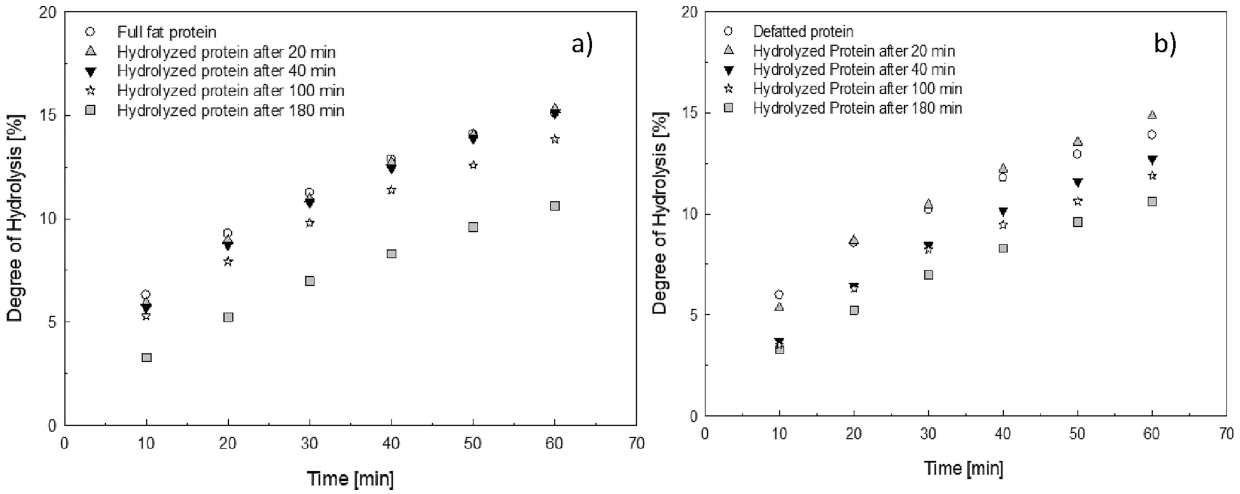

Figure 7. Effect of supplementing by 1% *w*/*v* the total initial protein concentration with hydrolysates harvested at different reaction times on the kinetics of hydrolysis of (a) full-fat and (b) defatted LFEY proteins.

Table 3. Hydrolysis Inhibition Ratios (eq 10) Obtained in Reactions by the Addition of 0.5 and 1% Hydrolyzed Protein Harvested at Different Times and Fat Concentrations[a]

	inhibition ratio IR_t (%)			
	0.5% hydrolysate		1% hydrolysate	
hydrolysate's harvest time	defatted LFEY	full-fat LFEY	defatted LFEY	full-fat LFEY
H_{20min}				3.50
H_{40min}			24.93	5.85
H_{100min}	10.91	10.91	26.30	14.43
H_{180min}	20.79	20.79	38.68	43.42

[a]LFEY: lecithin-free egg yolk.

effect of peptides with MW < 3 kDa in the hydrolysis of wheat proteins with Neutrase has been previously reported in the literature.[22]

5. CONCLUSIONS

The concentration of egg-yolk proteins and the defatting process of the LFEY substrate influenced the kinetics of the hydrolysis reaction with Alcalase 2.4 L. The Weibull equation showed that, despite the fact that the DH_{max} was similar in both substrates, the time needed to attain it was higher in the defatted LFEY. Accordingly, the Michaelis−Menten model showed that the K_M constant was higher for the hydrolysis of the defatted egg-yolk proteins (EYP). Hence, the enzyme had a greater affinity for the full-fat substrate probably due to conformational changes in egg-yolk protein molecules induced by the defatting process. Defatting also affected the hydrolysis reaction mechanism, that is, the enzyme follows the one-by-one reaction mechanism in the full-fat substrate and it changes to the zipper reaction mechanism in the defatted substrate. Consequently, the concentration and molecular weight profiles of peptides in hydrolysates from both substrates were different. The decrease in the degree of hydrolysis with increments in the initial protein concentration is attributed to a product inhibition effect mainly induced by low-molecular weight (1−2 kDa) peptides. This work gives insight into the factors that can affect the hydrolysis of egg-yolk proteins even though this raw material can vary from source/product to source/product.

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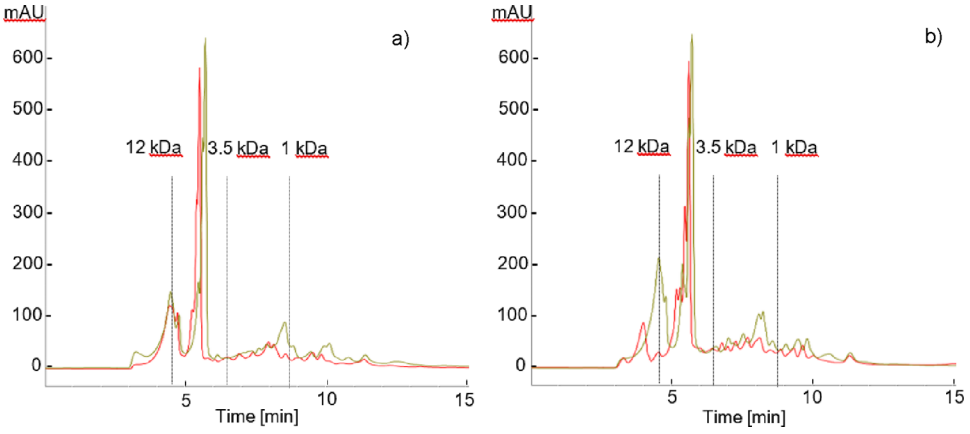

Figure 8. Molecular weight profiles of hydrolysates obtained after 20 min from the hydrolysis of (a) full-fat EY proteins and (b) defatted EY proteins with (green line) and without (red line) the addition of 1% *w*/*w* hydrolyzed proteins harvested from 180 min reactions and lyophilized. Total initial protein concentration: 10% *w*/*v*.

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Notes

The authors declare no competing financial interest.

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