Application of surface response methodology to optimize hydrolysis of wheat gluten and characterization of selected hydrolysate fractions

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Abstract

BACKGROUND: The aim of this work was to optimize the hydrolysis experimental conditions to obtain wheat gluten hydrolysates and to characterize fractions from hydrolysates with different hydrolysis degrees in order to develop protein functional ingredients. Surface response methodology was used to analyze the effect of reaction factors on the degree of hydrolysis to assess the conditions for maximum fungal protease enzyme activity. Hydrolysates having three different trichloroacetic acid indices (TCAI) were prepared. Soluble fractions at pH 4, 6.5 and 9 from these hydrolysates were characterized by electrophoresis, reverse-phase high-performance liquid chromatography, free amino group content and peptide chain length.

RESULTS: Temperature and pH ranges for highest enzyme activity at 2.5 h were 54–58 °C and 4.2–4.4, respectively. Hydrolysate fraction composition differs according to the hydrolysis degree and extracting pH, the difference being more pronounced at low TCAI. Hydrolysate having 32% TCAI is composed of peptides whose size is lower than 18.5 kDa, with an average peptide chain length of 14 amino acid residues.

CONCLUSION: The combination of hydrolysis degree and pH of extraction allows fractions of different peptide composition to be obtained, which could be taken into account when trying to find a defined composition related to determined functional characteristics.

Keywords: hydrolysis; wheat gluten; enzymes; experimental design; surface response methodology

INTRODUCTION

Gluten is one of the products obtained from the wheat wet milling process, which consists of several steps: hydration of flour, gluten formation, washing and drying the final vital gluten under mild conditions, which ensures that the unique viscoelastic properties are retained.

It is particularly used to improve commercial wheat bread flours of average quality and, also, to reinforce viscoelastic properties in special formulations[1,2] that require functional properties related to viscoelasticity or gluten vitality.[3] Due to the relative low price of gluten in comparison with other protein ingredients, there is interest in expanding the scope of gluten application by means of structural transformations which could provide other functional properties.

Hydrolysis is one of the alternatives that allow protein modification, which can be carried out either by chemical (acid or alkaline), physical, or enzymatic methods, the latter having remarkable advantages over the traditional chemical one.[4,5] Although different food-grade proteases can be used, the only information usually available is that offered by the manufacturer, which is limited to conditions of use based on tests carried out on a standard substrate, the structural characteristics of which differ from those corresponding to the protein system under study.

By controlling the reaction conditions during enzymatic hydrolysis, hydrolysates of various characteristics can be obtained. The degree of protein hydrolysis depends on the hydrolysate's expected use,[6,7] and low degrees of hydrolysis are required in order to maintain functional properties.[8,9]

The aim of the present work was to optimize the hydrolysis experimental conditions to obtain wheat gluten hydrolysates using surface response methodology and to characterize fractions from hydrolysates with different hydrolysis degrees in order to develop functional protein ingredients.

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EXPERIMENTAL

The enzyme used in the experiments, provided by Genencor SA (Arroyito, Córdoba, Argentina), is a fungal protease derived from *Aspergillus oryzae* (31 000 HU g^{-1}). The activity is expressed in hemoglobin units, where 1 HU is that activity which will liberate 0.0447 mg of non-protein nitrogen in 30 min under the conditions of the assay (denatured hemoglobin, pH 4.7; *T*: 40 °C). The enzyme is a mixture of endo/exopeptidases whose characteristics are as follows: effective pH 3.5–9, optimum pH 4.3–5; temperature range 30–50 °C.

Commercial vital gluten, provided by Molinos Semino SA (Carcarañá, Santa Fe, Argentina) was used as a substrate. Gluten composition was as follows: moisture 5.95% (AACC 44-15A method);[10] protein (N × 5.7) 77.20% dry basis (d.b.) (Kjeldahl–AACC 46-11 method);[10] starch 13.15% d.b. (Ewers polarimetric method); ether extract 0.71% d.b. (AACC 30-25 method);[10] and ash 0.834% d.b. (ICC No. 104–IRAM No. 15 851, standard technique).

Thermal treatment of vital gluten

In order to disperse vital gluten in water and secure a uniform suspension, a moderate thermal treatment was carried out. Vital gluten was placed in screw-cap cylindrical tubes (16 mm diameter) which were placed in a boiling water bath for 15, 25 and 35 min. The sample was then cooled by placing the tubes in a 21 °C water bath. To estimate the thermal treatment intensity, the impact on the viscoelastic properties of each sample was analyzed by means of an alveogram, carried out with a Chopin alveograph (Paris, France), which used a gluten–starch mixture as a model. The thermal treatment time was selected based on the capacity of the treated gluten to form a uniform dispersion, considering the alveogram as an indicator of thermal treatment intensity.

Effect of operating variables on degree of hydrolysis as studied by experimental designs

Surface response methodology was used to analyze the effect of reaction factors (pH, temperature and time) on the degree of hydrolysis with two experimental designs.[11] Experimental design 1, 'central composite blocked cube star', consisted of a total of 20 experiments which included 15 treatments with an additional five central points. Reaction factor levels were selected by taking into account the pH and temperature data supplied by the manufacturer, and the time, which ranged between 2 and 5 h, considering a time variation between each experimental level sufficient to show the difference between each treatment. In order to be more precise in the assessment of maximum enzyme activity for this particular substrate, a second experimental design was used, 'central composite design 2^2 + star', using pH (between 3 and 5) and temperature (between 35 and 55 °C) as independent variables, with a total of 11 experiments (nine treatments with an additional two central points). Time was kept constant (2.5 h). The data obtained were modeled by a second-degree polynomial function. In every case, hydrolysis was carried out in a laboratory batch system with temperature and agitation control. Protein concentration was 5% and enzyme/substrate (E/S) ratio 3%, pH being adjusted with HCl or NaOH, as necessary. A parallel reaction blank was carried out.

Hydrolysis reaction progress

The progress of hydrolysis was followed by means of the trichloroacetic acid index (TCAI), using TCA 20% and diluting the sample in a 1:1 ratio. N was measured by the Semimicro–Kjeldahl method. The TCAI, which was used as an indirect measurement of degree of hydrolysis (DH), was calculated as follows:

$$\begin{aligned}\text{TCAI} = [&\text{N soluble in TCA (hydrolysate)}\\ &- \text{N soluble in TCA (blank)}\\ &\times 100]/\text{total aminic N}\end{aligned}$$

Enzyme/substrate ratio

The conditions selected from the experimental design were pH 4.25 and temperature 55 °C and substrate concentration [S] was 5%. The E/S ratios under study were 1.87%, 3%, 3.75%, 5% and 10% (w/w). TCAI was determined by using the Lowry *et al*. technique[12] to measure protein concentration.

Preparation of hydrolysates

Hydrolysates were prepared in a 5 L batch reactor with agitation, using a thermostated bath kept at a constant temperature of 55 °C. HCl (3 mol L^{-1}) was added in order to maintain a constant pH of 4.25. A protein concentration of 8% (w/w) and E/S ratio of 5% were used. Hydrolysates were obtained at different reaction times: 31 min, 2 h and 6 h. Enzyme inactivation was carried out at 70 °C for 15 min. The hydrolysates were frozen at −20 °C and lyophilized.

Preparation of fractions at different pH

In order to obtain hydrolysate fractions at different pH (4, 6.5 and 9), a 2% (w/w, d.b.) solution of the different hydrolysates was prepared.[13] The pH was achieved by adding 0.8 mol L^{-1} HCl or 0.8 mol L^{-1} NaOH. The samples were stirred for 1 h at room temperature, and then centrifuged for 15 min at 8000 × *g* at room temperature. The supernatant (the extract at each pH) was frozen and protein content determined using the semimicro–Kjeldahl method.

Characterization of soluble fractions at different pH of thermally treated gluten (TTG) and hydrolysates

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (EF)

Electrophoresis was performed according to Laemmli,[14] in a 4–15% gradient buffer system using a

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Mini-Protean II electrophoresis cell (Bio-Rad, Hercules, CA, USA) with a Model 200/2.0 Bio-Rad source. The gel plates were fixed and stained with a solution containing 0.125% Coomassie Blue R-250, 50% methanol and 10% acetic acid in water. The pH extracts were mixed with sample buffer containing stacking buffer, glycerol and Bromophenol Blue, using a 1.33 dilution, with or without 5% β-mercaptoethanol. The mixtures were heated in a boiling bath for 90 s and loaded.

Reverse-phase high-performance liquid chromatography (RP-HPLC) of extracts

The extracts were diluted to a protein concentration ($N \times 5.7$) of 2.5 mg mL^{-1}. A Sephasil peptide C_8 column of 12 µm ST 4.6/250 (Pharmacia Biotech, Piscataway, NJ., USA) was used, together with an auto injector Waters 717 Plus Auto sampler (Millipore, Billerica, MA, USA), a Waters 600 E pump (multisolvent delivery system, Millipore), and a diode array detector (Waters 996, Millipore). Peptides were separated and eluted at 1.1 mL min^{-1}, at 60 °C, using the following buffers: buffer A: acetonitrile–water 2:98, with 650 µL L^{-1} trifluoroacetic acid (TFA); buffer B: acetonitrile–water 65:35 with 650 µL L^{-1} TFA, and detected at 210 nm. Since elution profiles of RP-HPLC can be grouped into categories according to increasing hydrophobicity of the eluted peptides,[15] chromatogram analysis was carried out by integrating peak areas in three sections of each chromatogram:

(a) components of low molecular weight and low hydrophobicity: 0–20 min of elution range;
(b) components of medium molecular weight and medium hydrophobicity: 20–40 min of elution range;
(c) components of high molecular weight and high hydrophobicity: 40–60 min of elution range.

Results were expressed as a percentage of each section with respect to the total area.

Determination of free amine group content

The *o*-phthaldialdehyde (OPA) technique[16] was used for this purpose. Free amine group content was used to calculate the number of peptide bonds cleaved during hydrolysis.

Estimation of peptide chain length (PCL)

PCL can be estimated by means of the following expression:[17]

$$\text{PCL (soluble fraction)} = 100 \times S/(h/h_{tot})\%$$

where h_{tot} is the total number of peptide bonds in the protein substrate (8.3 mEq g^{-1} protein), h is the number of peptide bonds cleaved during hydrolysis, and S is the fraction of soluble proteins.

Statistical analysis

Software Statgraphics Plus 3.0 was used for statistical analysis. The effect of variables on TCAI was analyzed by response surface methodology and the least significant difference (LSD) test was used to determine statistical differences among samples ($p < 0.05$).

RESULTS AND DISCUSSION

Thermal treatment of vital gluten

Table 1 shows the alveographic test results. A significant increase in gluten strength (P) is observed, even with slight thermal treatment (15 and 25 min). These results are in agreement with those reported by Jeanjean *et al*.[18] and Autran and Berrier.[19] Thermal treatment also produced a decrease in total absorbed energy (W) and in the swelling index (G). Values corresponding to 15 and 25 min samples were similar; however, a 35 min treatment proved excessive, which explains the P drop and also indicates a decrease in dough-forming ability. The increase in P/G ratio at the beginning of the thermal denaturation process is related to the decrease in cohesivity. These results indicate that the decrease in gluten-forming ability caused by heat treatment is better described by G and W. Gluten treated for 25 min produced an alveographic diagram similar to that frequently observed in wheat samples taken from silos with overheating.[20] The loss of gluten bread-making quality could be associated with the formation of aggregates between the different protein fractions,[21] which could be the result of polymerization by sulfhydryl–disulfur interchange.[22] The selected thermal treatment was 25 min, since better gluten dispersion was obtained when compared with that corresponding to 15 min, which seems to be insufficient for an easy dispersion, and also with 25 min treatment gluten is not over-treated as in the case of 35 min treatment.

Effect of operating variables on degree of hydrolysis as studied by experimental designs

Table 2 shows the results of TCAI corresponding to the central composite blocked cube star experimental design. It is observed that the highest TCAI value was obtained for one extreme of the design (pH 3.8 and 40 °C) and the lowest ones were around pH 6.5

Table 1. Alveographic test

Sample	*W*	*P*	*G*	*P/G*
Vital gluten	172c	82b	15.5c	5.29a
15 min gluten	135b	111c	11b	10.1c
25 min gluten	135b	119d	10b	11.9d
35 min gluten	35a	71a	8.5a	8.35b

W, total absorbed energy; *P*, strength; *G*, swelling index; *P/G*, alveographic equilibrium index.
For each column, different letters represent significant differences between the samples ($p < 0.05$).

Table 2. Results of TCAI corresponding to the central composite blocked cube star experimental design. [S] = 5%, E/S = 3%

Experiment no.	pH	Temp. (°C)	Time (h)	TCAI
1	6.5	40	3.5	3.29
2	8.0	32	2.0	0.44
3	8.0	32	5.0	1.05
4	5.0	48	2.0	11.20
5	5.0	48	5.0	17.00
6	6.5	40	3.5	2.60
7	5.0	32	5.0	9.65
8	8.0	48	2.0	0.70
9	8.0	48	5.0	1.35
10	5.0	32	2.0	4.62
11	6.5	40	3.5	2.42
12	6.5	40	3.5	2.22
13	6.5	25.5	3.5	1.07
14	9.2	40	3.5	0.90
15	6.5	54.5	3.5	5.30
16	6.5	40	3.5	2.85
17	3.8	40	3.5	17.08
18	6.5	40	6.21	3.56
19	6.5	40	0.79	0.73
20	6.5	40	3.5	2.96

or higher. The Pareto chart (Fig. 1) shows that pH is the variable of highest impact, both in its linear and quadratic term. The effects of the three variables were significant, particularly in the case of the linear terms, the pH quadratic term and the interactions pH × temperature and pH × time. The time quadratic term and interaction time × temperature, on the contrary, were not significant ($P < 0.05$). Figure 2 shows the response surface for TCAI as a function of pH and temperature, for 3.5 h. A minimum TCAI was observed around 6.5–7.5 and low temperature. Bombara,[23] working with wheat flour, reported similar TCAI values at 54 °C, pH 6.5 and time less than 6 h. However, Adler-Nissen,[17] working with soy protein and an enzyme derived from *A. oryzae*, found that the DH obtained at pH 7 was similar to that at pH 5. In the present work, the TCAI obtained for pH 7 is surprisingly lower than that at pH 5, this difference being probably due to the fact that experiments were carried out with different substrates and also a different method of measuring DH. Gluten isoelectric point is around pH 7, which favors molecular association by hydrophobic interactions, and makes them less susceptible to enzymatic attack by aggregate formation. According to the results obtained, the highest TCAI values will be obtained in the pH range between 3.7 and 5.

Table 3 shows the results of TCAI corresponding to the central composite design 2^2 + star. Analysis of variance showed that the two variables were significant in every term and that the correlation coefficient was $r = 0.9907$. Figure 3 shows the surface response for TCAI as a function of pH and temperature, and shows a maximum around pH 4.2–4.4 and at 54 and 58 °C.

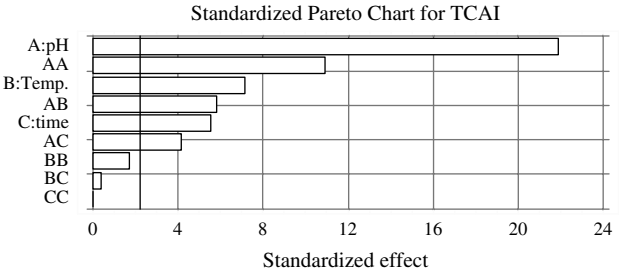

Figure 1. Pareto chart corresponding to central composite blocked cube star experimental design. [S] = 5%, E/S = 3%; TCAI, trichloroacetic acid index.

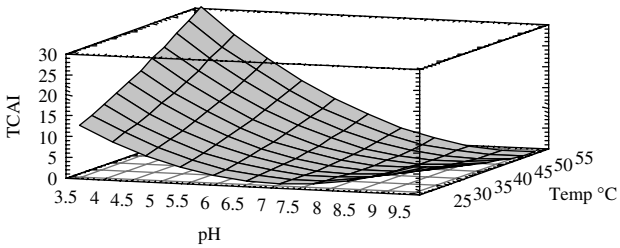

Figure 2. Surface response for TCAI as a function of pH and temperature, for 3.5 h, corresponding to central composite blocked cube star experimental design. [S] = 5%, E/S = 3%; TCAI, trichloroacetic acid index.

Table 3. Results of TCAI corresponding to the central composite experimental design 2^2 + star. [S] = 5%, E/S = 3%

Experiment no.	pH	Temp. (°C)	TCAI
1	4.25	45	17.600
2	3.0	35	12.484
3	3.0	55	12.630
4	5.5	35	3.886
5	5.5	55	13.568
6	4.25	45	17.466
7	4.25	30.9	8.500
8	4.25	59.1	20.036
9	2.48	45	9.060
10	6.02	45	3.860
11	4.25	45	17.000

The polynomial corresponding to this experimental design was

$$\text{TCAI\%} = -46.5172 + 19.1734 \times \text{pH} + 0.842979 \times \text{Temperature} - 3.44202 \times \text{pH}^2 + 0.19072 \times \text{pH} \times \text{Temperature} - 00\,147\,418 \times \text{Temperature}^2$$

According to the information given by the manufacturer, the operating conditions (pH and temperature) of this enzyme include conditions at which very low activity was observed in the present work. This underlines the importance of checking the operating conditions for each particular enzyme–substrate system; surface response methodology could be used to find the best conditions.

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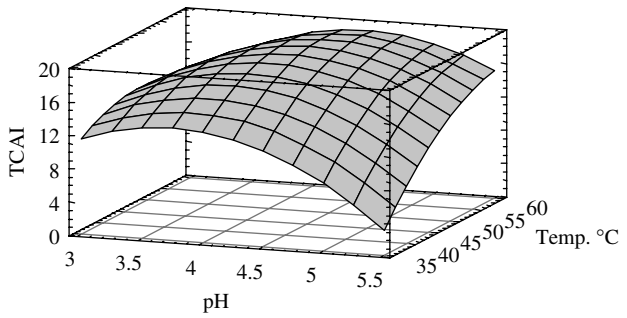

Figure 3. Surface response for central composite experimental design 2^2 + star. [S] = 5%, E/S = 3%; TCAI, trichloroacetic acid index.

Selection of E/S ratio

Figure 4 shows the hydrolysis curves corresponding to different E/S ratios at pH 4.25 and 55 °C, expressed as percentage (%w/w). It is observed that TCAI increases with an increase in E/S. However, when selecting the enzyme concentration to reach a particular degree of hydrolysis, the relationship effectiveness–economy has to be taken into account. A higher enzyme concentration could be used in order to increase hydrolysis rate, but in this case a cost analysis of raw material *versus* reaction rate should be carried out. In our case, a 5% E/S ratio was chosen because it was considered to be a reasonable ratio, although with products having a high added value (for example, peptides with specific functions) a higher E/S ratio could be selected.

Analysis of hydrolysates and solubility

Three hydrolysate samples were prepared under the conditions previously selected (pH, temperature and E/S) for times 0.5, 2 and 6 h and their TCAI were 14%, 22% and 32.6%, respectively.

Table 4 shows the protein solubilities corresponding to thermally treated gluten and the three hydrolysates. At pH 6.5 (isoelectric point region), hydrolysate solubility was always lower than that corresponding

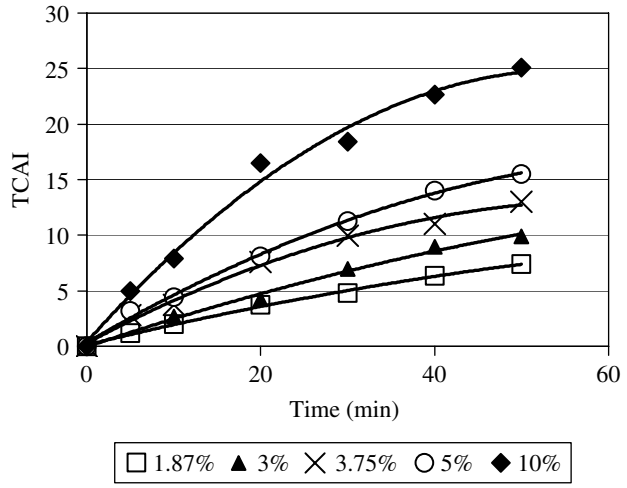

Figure 4. Effect of E/S ratio (%w/w) on TCAI; [S] = 5%, pH = 4.25, T = 55 °C; TCAI, trichloroacetic acid index.

Table 4. Solubility of gluten hydrolysates and thermally treated gluten (TTG)

Samples	pH	$x \pm$ SD
TTG	4.0	58.04 ± 0.41d
	6.5	5.88 ± 0.13h
	9.0	25.58 ± 0.88g
TCAI 14%	4.0	80.47 ± 0.28a,b
	6.5	35.87 ± 0.06f
	9.0	57.77 ± 0.37d
TCAI 22%	4.0	81.01 ± 0.50a
	6.5	53.74 ± 0.32e
	9.0	73.88 ± 0.63c
TCAI 32.6%	4.0	77.58 ± 0.16a,b
	6.5	67.54 ± 0.69b,c
	9.0	79.94 ± 0.06a

Different letters represent significant differences between the samples ($P < 0.05$); $x \pm$ SD, mean of triplicate ± standard deviation.

to other pH values. The enzyme could hydrolyze the protein, producing large-size polypeptides, whose solubility is more pH dependent, together with small peptides. The pH dependence could be associated not only with the size of the peptides produced by hydrolysis but also their net charge. Linares *et al*,[15] working with wheat gluten hydrolysate (DH 1.4%, measured as the increase in free amino groups), observed that a pH increase from 4 to 6.5 produced a decrease in the solubility of the more hydrophobic peptides. Molecular size decrease allows hydrolysates to be obtained which are more soluble than the native proteins and within a wider pH range. The solubility profile of the partially hydrolyzed protein improves along the whole pH scale, being generally higher with higher TCAI, since the protein does not form large aggregates even at the isoelectric pH. The solubility increase obtained from a limited proteolysis is attributed to the formation of either smaller and more hydrophilic polypeptide unities or soluble hydrophobic peptides,[24] as well as the disruption of protein aggregates.[25]

Characterization of soluble fractions at different pH of thermally treated gluten (TTG) and hydrolysates

SDS-PAGE

When observing the electrophoresis pattern corresponding to non-hydrolyzed gluten (TTG) (Fig. 5(a), lanes 4, 5 and 6), without mercaptoethanol (ME), a diffuse stain can be observed for the extracts obtained at the three pH values. These could be attributed to the presence of protein aggregates distributed along the range of the molecular weights (MW) under study. When using ME (Fig. 5(b), lanes 4, 5 and 6) these aggregates break up and form well-defined bands, which show that disulfur bridges may be involved in their formation. Moreover, pH has a marked influence on the fractions of solubilized proteins. The extracts at pH 4 and 9 treated with ME (Fig. 5(b), lanes 4 and 5) are formed by components of MW higher than 94

and lower than 14.4 kDa, and the extract at pH 6.5 (Fig. 5(b), lane 6) is mainly formed by components whose MW is around 43 and 14.4 kDa.

The differences between the fractions extracted at pH 4 and 9 are particularly evident at MW ranging between 67 and 30 kDa. According to Cornec *et al*,[26] gliadins are present between fractions of 30–40 kDa, except for ω-gliadin, which is present in the fractions rich in glutenins. Glutenins of high MW are present in fractions of more than 50 kDa and those of low MW are present within the range 40–43 kDa. Fractions lower than 20 kDa are non-storage proteins, but membrane and lipid binding proteins.

The extracts at pH 9 and 4 from hydrolysate with TCAI = 14.1% (Fig. 5(a), lanes 1 and 3, respectively) have components of MW higher than 43 kDa which, when treated with ME (Fig. 5(b), lanes 1 and 3), show that they corresponded to subunits of MW below 43 kDa, linked by disulfur bridges. That is, with a low hydrolysis, the extracts at these pH showed components of high MW, which corresponded to glutenin fractions associated with S–S, though already hydrolyzed. The extracts at pH 4 and 9 have different components between 40 and 30 kDa. In the case of the pH 6.5 fraction (Fig. 5(b), lane 2) the extracted polypeptides are mainly formed by monomers of MW below 20.1 kDa, though showing an MW dispersion, like those components of extracts at pH 9.

The extracts from hydrolysates with higher TCAI (22% and 32.6%) had components whose MW are higher than 43 kDa (electrophoresis without ME is not shown), which correspond to S–S bridge associations of peptides whose MW is lower than 43 kDa for TCAI 22% and lower than 20.1 kDa for TCAI 32.4%. This is shown in Fig. 6 for samples treated with ME. In every case there seem to be components of 14.4 kDa, which do not hydrolyze, since there is always a band corresponding to that MW.

The extracts at pH 4 and 9 are similar but differ from that at pH 6.5, the later lacks the components corresponding to the 20–40 kDa zone (ITCA 32.6%) or have low quantities of them (TCAI 22%), this probably being related to the presence of a limiting peptide size for the enzyme action in the range of hydrolysis degrees evaluated in this work.

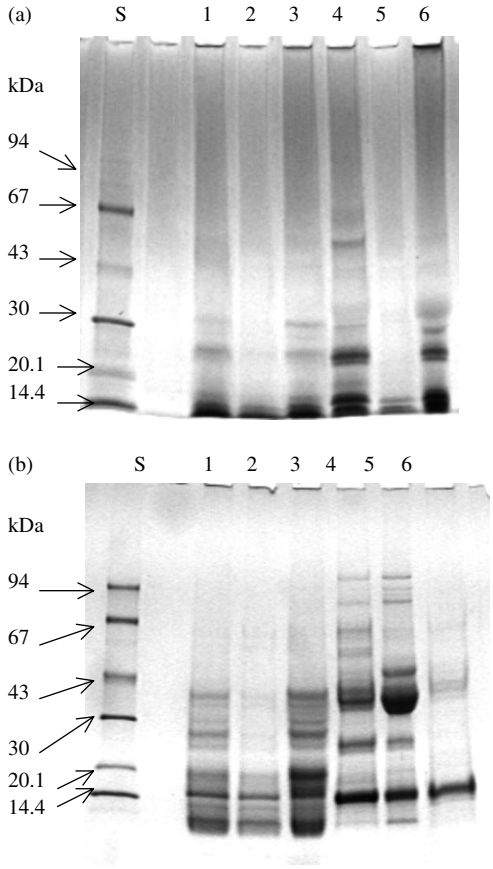

Figure 5. (a) EF-SDS-PAGE without mercaptoethanol of pH extracts from thermally treated gluten (TTG) and hydrolysate (TCAI: 14%). S, standard; 1, 14%–pH 9; 2, 14%–pH 6.5; 3, 14%–pH 4; 4, TTG–pH 9; 5, TTG–pH 6.5; 6, TTG–pH 4. (b) EF-PAGE-SDS with mercaptoethanol of pH extracts from TTG and hydrolysate (TCAI: 14%). S, standard; 1, 14%–pH 9; 2, 14%–pH 6.5; 3, 14%–pH4; 4, TTG–pH 9; 5, TTG–pH 4; 6, TTG–pH 6.5.

Average peptide chain length

Figure 7 shows the average peptide chain length of the soluble fraction. The extracts at pH 6.5 for the same TCAI have the peptides with the smallest sizes. For the hydrolysate with 32.6% TCAI, protein extractability was not much affected by pH, and their extracts have peptides of about 14 amino acid residues.

The way the enzyme acts can be seen with the PCL measured at the pH corresponding to the isoelectric point (pH 6.5) or pH 9, where the solubility of the thermally treated gluten is low. PCL shows a rapid decrease with progress of the enzymatic reaction during the first 30 min. This increase is then slower, which shows that soluble peptides are degraded during hydrolysis until they reach a certain length. After that, the enzyme would not act on them but on the substrates of higher MW, which increases solubility.

Integrated areas of RP-HPLC

Figure 8 shows the integrated areas of RP-HPLC chromatograms. The extracts at pH 6.5 have a higher

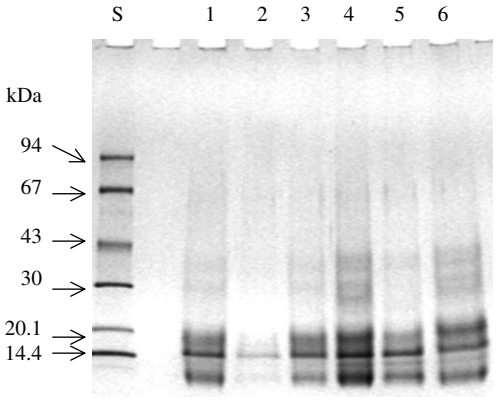

Figure 6. EF-PAGE-SDS with mercaptoethanol of pH extracts from hydrolysate (TCAI: 22% and 32.6%). S, standard; 1, 32.6%–pH 9; 2, 32.6%–pH 6.5; 3, 32.6%–pH 4; 4, 22%–pH 9; 5, 22%–pH 6.5; 6, 22%–pH 4.

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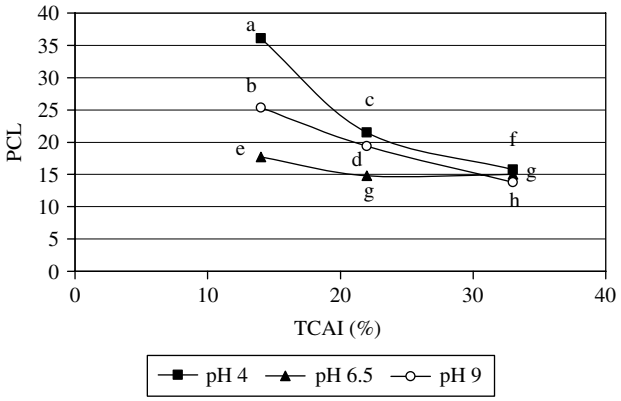

Figure 7. Average peptide chain length *versus* TCAI. Different letters represent significant differences between the samples ($P < 0.05$).

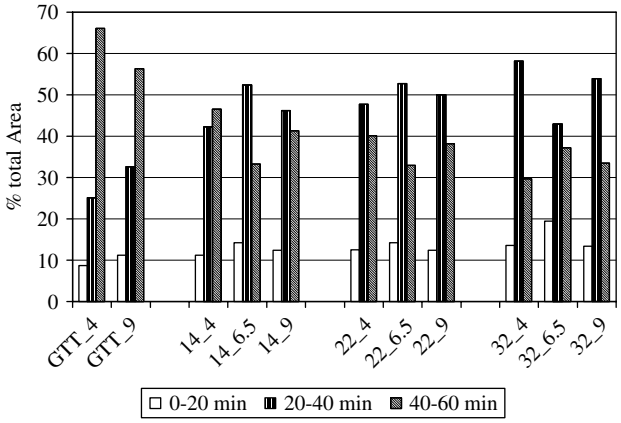

Figure 8. Area percentage of each eluate section (0–20, 20–40 and 40–60 min) with respect to the total area, corresponding to different extracts at pH 4, 6.5 and 9 of hydrolysates (TCAI: 14%, 22% and 32.6%) and thermally treated gluten (TTG).

content of components with low hydrophobicity and MW (0–20 min zone) compared to the other extracts, which is in agreement with the lower PCL values obtained.

As TCAI increases, the quantity of components with average hydrophobicity and MW (20–40 min zone) increases, except at pH 6.5, where it decreases. It is also observed that as TCAI increases there is a clear decrease in hydrophobic components (40–60 min zone), though there are practically no changes for pH 6.5 extracts. This could be attributed to the break pattern of this protease. Since hydrolysis products have components of high and low MW, at pH 6.5 the solubilized proteins would be poorer in hydrophobic components of high MW, and richer in components of low hydrophobicity, which is reversed at TCAI 32.6%, when solubility increases. It is then confirmed that during the first reaction stages (TCAI 14% and 22%) this enzyme hydrolyzes the protein, generating components of high MW, the solubility of which is more pH dependent, together with soluble components of low MW. At TCAI 32.3%, the enzyme generates peptides which are soluble (solubility higher than 76%) and less pH dependent.

These results suggest that enzyme action on gluten proteins reduced the size of gliadin and glutenin molecules. The resulting hydrolysate varied in composition with the degree of hydrolysis reached, though in general peptide size distribution would not be unimodal. At TCAI 32%, most of the hydrolysate is composed of peptides whose size is less than 18.5 kDa, with an average PCL of 14 amino acid residues. PCL is useful to evaluate the hydrolysis process at pH values at which the proteins have a low solubility. However, it is important to point out that PCL does not represent the average of a unimodal population, particularly at low degrees of hydrolysis.

CONCLUSIONS

Surface response methodology was used to find the experimental conditions required for the highest enzymatic activity for hydrolysis of wheat gluten by fungal protease.

Fractions at different pH values had different compositions, mainly at low TCAI (14% and 22%), which could be taken into account when trying to find a defined composition related to determined functional characteristics.

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